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DAILY MEAL PATTERNS, VOLUNTARY FOOD INTAKE AND FATTENING OF REINDEER DURING WINTER AND RESPONSES TO INSULIN

A

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Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

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DOCTOR OF PHILOSOPHY

By

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General Matriculation, D.V.M., M.S.

Fairbanks, AK

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DAILY MEAL PATTERNS, VOLUNTARY FOOD INTAKE AND FATTENING OF REINDEER DURING WINTER AND RESPONSES TO INSULIN

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Abstract

I determined the effect of insulin injections on daily feeding behavior and voluntary food intake (VFI) in reindeer (*Rangifer tarandus t.*) fed a concentrate ration during winter. Food intake in the absence of insulin injections was down regulated and characterized by small, regular meals during daylight and irregular and sometimes large nighttime meals. Each large nighttime meal was associated with a long post-meal interval. Daytime meal size could be predicted from an estimate of the energy deficit incurred since the previous meal; however, the occasional oversized nighttime meals were not predicted from energy deficit and suggested that appetite may be deregulated at night. I hypothesized that a low daily dose of long acting insulin (1.0 IU/kg BW, s.c.) would result in regular feeding day and night, which should result in reduced VFI. Changes in serum insulin concentration could not be detected following insulin treatment, however exogenous insulin resulted in a loss of daytime and nighttime differences in meal size and intermeal interval length and a decrease in mean daily meal size. Over a 21 d treatment period, exogenous insulin prevented an increase in VFI during a warming trend and tended to counter a linear decline in body mass and backfat depth (measured by ultra-sound) typified by control animals (given Lactate Ringer 0.005 ml/kg BW, s.c.). The influence of insulin over fat retention suggests that reindeer are capable of lipogenesis in winter. A combination of rhythmic variation in satiety response to meals during daylight and decoupling of meal size and frequency at night is suggested as an endocrine model underlying daily appetite regulation in the reindeer.

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This thesis is dedicated to Berkeley, 1987-2000.

General Introduction

During winter, voluntary food intake (VFI) decreases to approximately one third of maximum summer intake in many north temperate ungulate species (McEwan and Whitehead 1970; White et al. 1984; Larsen et al. 1985). VFI and daily dry matter intake (DDMI) are a function of the number of meals per day, the respective inter-meal intervals and individual meal sizes. Variation in temporal organization of DDMI in winter should be due to changes in hormone levels of appetite regulators, food availability, food quality, and animal-animal interactions. Daily feeding activity patterns show rhythmicity, depending on whether a species is mainly nychthemeral (poly-cyclic across 24-h), twilight active (crepsicular), active at sunrise and sunset (crepuscular), or active diurnally or nocturnally in its feeding behavior (Errikson et al. 1981; Senn et al. 1990; Sibbald 1994; Maier and White 1998; Colman 2000). Feeding behavior can be modulated by photoperiod, which is transduced within the organism via amplitude and duration of nocturnal secretion of the pineal hormone melatonin (Eloranta et al. 1992; Loudon 1994). An important aspect of the level of VFI by north temperate ruminants in winter is the relatively tight linkage between energy intake and energy requirements. Under *ad libitum* feeding, body mass undergoes only a modest decline throughout winter (Ryg 1983; Suttie et al. 1991). Although feeding activity patterns are well documented (Eriksson et al. 1981; Maier and White 1998), the actual size of meals and physiological controls over meal size in relation to DDMI are not well known in north temperate deer. From studies with laboratory animals it is known that alimentary stretch receptors and hormones control the end of one feeding event and the initiation of the next (Bray 2000ab; Szekely

2000: Forbes 1996). The list of hormones that have been shown to influence VFI and individual meal size and frequency in laboratory animals is extensive and includes insulin, glucagon, leptin, growth hormone, thyroid hormone, glucocorticoids, corticotropin releasing hormone, and gonadal steroids (Bray 1985; Bray and York 1998; Bray 2000ab; Cavagnini et al. 2000; Mystkowski and Schwartz 2000).

How hormonal control influences feeding behavior in a species adapted to limited food resources is not known. Of the hormones postulated to affect food intake and meal patterns, insulin interacting with glucose concentration (the glucostatic theory of intake regulation) (Mayer 1953; 1996) has been shown to exhibit pronounced seasonal fluctuations in north temperate ruminants (Larsen et al. 1985). In reindeer, serum insulin concentration peaks in summer during a time of hyperphagia and maximum body mass gain and is lowest during winter hypophagia and weight stasis or loss (Larsen et al. 1985). In these studies, the role of feeding behavior was not studied. Summer hyper-insulinemia could be a product of hyperphagia, however this hypothesis has not been tested in reindeer. Evidence exists for other species (humans, Boden et al. 1996; pigs, Thaela et al. 1995; quails, Tedford and Meier 1993) that daily (circadian) and ultradian (< 24 h) fluctuations of plasma insulin occur that are not only due to the rhythm of feeding behavior; this was demonstrated in animals deprived of feed and in isolated perfused pancreatic tissue (Peschke and Peschke1998). These findings argue for the existence of an endogenous pacemaker. Taking these findings together, I propose that daily food intake regulation in reindeer could be a product of daily serum insulin fluctuations with insulin affecting both meal size and frequency. Investigations described within this thesis

were conducted to further our understanding of feeding behavior and short-term regulation of intake in reindeer during winter, when appetite is highly regulated and insulin levels are at their annual lowest; and to clarify insulin's role in governing daily meal patterns, daily VFI and subsequent effect on body mass and body fat. I required an accurate non-invasive measure of body fat in live reindeer to make repeated measures on body fat reserves in order to evaluate VFI and body mass changes. In reindeer a linear relation between backfat depth (BFD) and total body fat indicates (Reimers & Ringberg 1983; Adamczewski et al.1987; Chan-McLeod et al.1995) that BFD measured *in vivo* could be a useful research tool to estimate total body fat. Body fat also can be estimated from a body condition score (BCS) (Gerhart et al. 1996). In chapter 1, I evaluate the feasibility of using portable real-time ultrasound to measure BFD in live reindeer. Ultrasound has been used to measure back fat depth in domestic livestock in order to predict carcass chemical composition (Fisher 1997). Comparing total body fat predicted from BFD with those values estimated from BCS tested efficacy of the technique. No bias was found in the prediction of body fat between techniques. Therefore, the estimates of BFD provided the tool to give a non-invasive estimate of body condition of live reindeer.

Daily distribution of meal size and the relation of meal size to VFI have not been measured in reindeer. The only reports on meal size involve studies on the influence of food structure, biomass and density on eating behavior of the grazing animal (White and Trudell 1980; Trudell and White 1981). I needed a simpler system and chose to determine meal size by measuring the intake of food from a continuous record of the

mass of a feed bin on a scale (Chapter 2). I determined feeding behavior and meal pattern of reindeer during winter by assessing the daytime and nighttime distributions of meal size, and inter-meal interval (Baile 1975; Sibbald 1994). By monitoring trends with time in feeding behavior and meal patterns I determined the influence over VFI, body mass and body fat. In sheep fed high quality diets (chopped hay-concentrate of > 65% apparent dry matter digestibility) at a level where body mass and body fat remain constant (i.e., maintenance), meal size up to 300 g is correlated with the pre-meal interval, but the post-meal interval is not correlated with the meal size (Baile 1975). In the ruminant, a tight linkage of meal size and pre-meal interval is an indicator that energy need (i.e., energy deficit) is met by intake (i.e., by metabolic control). Thus Baile's data support some form of metabolic control over meal size rather than a physical constraint imposed by the rate of processing and passage of food particles (i.e., rumen fill) (Campling 1970).

To examine metabolic and physical factors involved in short-term intake regulation in the reindeer I estimated the expected energy deficit incurred since the previous meal and determined whether the energy deficit was related to the metabolizable energy ingested (MEI) in the meal. Alternately I tested the hypothesis that meal size was related to a deficit in rumen fill. A tight linkage of MEI and energy deficit is proposed to involve a glucose-insulin axis (Mayer 1953; Mayer 1996). According to the Mayer model (the glucostatic theory of intake regulation) energy intake in relation to energy needs is under glucostatic control. Transient excursions in blood glucose concentration function as on-off switches of feeding behavior, (i.e., declines in blood glucose concentration result in meal onset). Therefore, the role of glucose and thus insulin in

control of food intake is dynamically functioning as a satiety factor and an initiation signal. Mayer's model of short-term intake regulation does not involve any of the more recently discovered central and peripheral physiological correlates of feeding behavior particularly the neuropeptide systems (Mondal et al. 2001; York 1999). Thus, the glucostatic model can be criticized for being too simplistic; however, in lieu of the seasonal fluctuations of insulin levels and the tight linkage of energy intake to requirements in reindeer during winter, evaluation of the glucostatic model appears applicable for reindeer.

Based on results from Chapter 2 that daytime meals were related to the pre-meal interval, with a tight coupling of MEI to energy deficit and maintenance of meal-to-meal energy balance, I suggested a regulative role for insulin over intake. Since the nighttime meals were large and unpredictable I hypothesized that control was deregulated at night. I was uncertain as to whether insulin could be initiating feeding events through an endogenous rhythm or responding to food ingestion. In Chapter 3, I determined whether a periodicity of serum insulin secretion occurred in the absence of feeding i.e. during fasting. Further, I determined whether there were temporal inter-relations of serum insulin levels with serum glucose and lactate concentrations during an 18 h fast. A rhythmic insulin pattern was found and since these oscillations were not correlated with those for serum glucose and lactate, I hypothesized that oscillations in insulin secretion could be playing a role in initiating the next meal.

Chapter 4 is a test of the hypothesis that insulin regulation of feeding cycles is decoupled during nighttime feeding (Chapter 2). I daily injected a group of five reindeer

with an insulin dose (1 IU/kg) for 21 days and compared their feeding behavior with a control group of five animals given saline. I predicted that insulin treatment would improve glucose and volatile fatty acid (VFA) absorption and utilization (i.e. oxidation and storage), thereby reducing inter-meal interval, and increase meal frequency, particularly at night but also during daytime. I hypothesized that for nighttime meals the elevated insulin levels would restore insulin regulation of feeding cycles resulting in more regular feeding and a decrease in meal size. Therefore, differences between daytime and nighttime meal patterns should diminish possibly resembling a nychthemeral feeding activity pattern. Furthermore, the reinforcement of a metabolic control by insulin during nighttime should result in meal MEI being equal to energy deficit during nighttime as it is during daytime. In Chapter 4, I report support for the hypothesis that insulin is involved in meal size regulation since regular meal eating was maintained day and night by insulin injections, and differences between daytime and nighttime meal patterns disappeared. However, serum insulin levels did not differ significantly between control and treatment groups. I interpreted the absence of a significant effect on serum insulin levels that either the insulin dose was cleared from blood in less than 24 h or that exogenous ultralente insulin suppressed endogenous insulin secretion, thus resulting in no net effect on concentrations. Corroborative evidence from human and laboratory studies shows suppression of endogenous insulin secretion by exogenous insulin injections (Beischer et al. 1979). However, in humans ultralente insulin does not suppress meal elicited insulin secretion, nor does it suppress weight gain (Coutant et al. 2000). Therefore, I concluded that lack of a clear effect of ultralente insulin on serum insulin levels in this study did not

invalidate further analysis of insulin treatment on feeding variables. I believe it is justified to associate the observations on nighttime feeding and opposing trends in DDMI and increasing BFD (lipogenesis) of the treatment group within the study to the insulin injections. Based on the findings from the insulin injection study, I proposed that daytime and nighttime differences in feeding behavior are a result of a combination of altered insulin secretion and sensitivity to insulin. Since the main effects of feeding behavior were restricted to nighttime feeding, I speculated that melatonin was responsible for decoupling meal interval-meal size relationships at night; i.e. the animal becomes less responsive to serum insulin concentration. A combination of rhythmic variation in satiety response to meals during daylight and decoupling of meal size and frequency at night is suggested as an endocrine model underlying daily appetite regulation in the reindeer. Such a model should be adaptive in north temperate species as it allows for fewer feeding events at night and should lower daily maintenance energy requirements during winter.

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Chapter 1

Assessment of body fat dynamics in reindeer using real-time ultrasound[1]

Abstract

The relation between backfat depth (BFD) and total body fat in reindeer is linear, suggesting that BFD measured *in vivo* could be a useful research tool to estimate total body fat. We evaluated the feasibility of using portable real-time ultrasound to measure backfat depth in live reindeer. In a group of nine non-pregnant female reindeer we estimated maximum backfat depth (BFD), body mass (BM, kg) and an indirect measure of body fat, the body condition score (BCS) every 2 days for 14 d from the start of the study on December 6. The different layers of tissue between skin surface and muscle could be visually distinguishable by echogenicity. Ultrasonographically, skin and fascia appeared as a white hyperechogenic band, subcutaneous fat appeared uniformly gray and muscle was dark. The range of maximum BFD on day 1 was 3.10-5.70 cm and 2.70-5.40 cm on day 14. Mean BM declined significantly ($P < 0.001$) and the decline in mean BFD was marginally significant ($P = 0.055$). BCS and body reserve index (BRI = BCSxBM) were both correlated with maximum BFD ($r^2 = 0.61$; $r^2 = 0.75$). The results suggest that portable real-time ultrasonography is a useful field technique to estimate subcutaneous backfat depth in reindeer, which alone or in combination with measures of BM and BCS can be used to monitor body condition.

[1]prepared to be submitted to Rangifer as Stimmelmayr, R. & White, R.G. Assement of body fat dynamics in reindeer using real-time ultrasound. 00:00-00.

Introduction

Body composition, an important indicator of nutritional status and energy reserves of reindeer and caribou (*Rangifer tarandus L.*), is mainly a reflection of their fat reserves. Fat reserves are generally assumed to determine an individual's reproductive potential as well as it survivability (Gerhart et al., 1996). Total dissectible and chemically determined body fat are tightly related to depth of back fat in Rangifer (Reimers & Ringberg, 1983; Adamczewski et al., 1987; Chan-McLeod et al., 1995). Therefore, measurement of subcutaneous back fat depth is a commonly used indirect method for estimating total body fat. Ultrasound has been used to measure back fat depth in live animals in order to predict carcass chemical composition in livestock (Fisher, 1997). We evaluated the feasibility of using portable real-time ultrasound to measure subcutaneous back fat in captive reindeer.

Materials and methods

Nine non-pregnant adult female reindeer were used for the 14 d study. Mean ± SEM body mass (BM) at the beginning of the study (6 December) was 103 ± 3.5 kg with a range of 89 - 116 kg. Animals were held at the Large Animal Research Station, Fairbanks, Alaska (64° 52' 39" N, 147° 49' 29" W) and had free access to a standard pelleted ration (UAF-RR, Alaska Garden and Pet Supply, Anchorage, AK 99510). Snow was freely available. A real-time portable scanner (Technicare Model 210DX) with a linear 5 MHz transducer was used to measure maximum backfat depth (BFD) along a longitudinal line between the tuber coxae and the tuber ischii as described by Stephenson

et al. (1993). Sampling interval was alternate days. Before scanning, the animal's hair was clipped and shaved wide enough (2.5 cm) along the longitudinal line to permit scanning of the region. To reduce the risk of frostbite shaved areas after scanning were covered with gauze strips and strip secured to the animals hair coat using clips. Vegetable oil was applied as contact medium. All examinations were performed on standing animals. The echogenicity of skin, subcutaneous fat, fascia and muscle tissue was assessed and compared. Maximum BFD was measured with electronic calipers to the nearest 0.1 cm at the point of maximum thickness along the line. To judge the reproducibility and reliability of the results the inter-assay variation coefficient was calculated after 10 examinations. All animals were weighed daily (nearest 0.5 kg). During measurements animals were restrained in a stansion fitted with a neck halter. Body condition scores (BCS), estimated as the sum of ranks from 1 (emaciated) to 5 (obese) of soft tissue covering bone as assessed by palpation at three sites on the animal: withers, ribs, and rump (Gerhart et. al., 1996), was measured on alternate days. The product of BCS and body mass (BM), termed the body reserve index (BRI) was used to estimate the ingesta-free body fat (IFBF, kg) by the equation given by Gerhart et al. (1996): LN (IFBF) = 1.444*LN (BRI)-7.713. Dissectible fat content (Y, kg) was estimated at 56% of BM (Z, kg) (Ringberg et al., 1980) and BFD (X, cm) using the equation of Reimers and Ringberg (1983): $Y = -3.5371 + 0.0990\ X + 0.0014\ X^2 + 0.2093\ Z$. Fat content (Y, kg) was estimated by the equation given by Adamczewski et al. (1987): $Y = -0.714 + 0.629^*\ X$, where X = BFD. Percent body fat (Y, %) was estimated by the equation given by Chan-McLeod et al. (1995): $Y = (\sin [0.2804 + 0.0498\ (X)])^2 \times 100$, where X = BFD.

Repeated measurement ANOVA was used to test for a significant difference of BFD, BM, BRI, and BCS over time (SAS 6.12 Windows). Pierson correlation coefficient between maximum BFD and BM, BCS, and BRI were computed. Relations between variables were determined with linear regression using only day 1 measurements (n = 9). Significance level was set at $P < 0.05$.

Results

The different layers of tissue could be visualized and were easily distinguishable by their different echogenicity (Fig.1). Ultrasonographically the skin and the fascia appeared as white, hyperechogenic narrow bands. The subcutaneous fat layer appeared uniformly gray and was less echogenic. Transient shifts in echogenicity of skin and fat layer were observed making distinction between the two layer interface difficult. Packing the rump region with snow for a period of 10 minutes resulted in an increased echogenicity of the fat layer and a homogenous appearance of both tissue types. The effect was reversible. The internal muscle layer was easily distinguishable from the adjacent fat layer by the overlaying white fascia. Muscle tissue was overall dark in appearance (hypoechogenic) with multiple hyperechogenic areas scattered throughout the proximal portion of the tissue.

Maximum BFD was located 2/3 along the longitudinal line between the tuber coxae and the tuber ischii. Inter-assay variation, coefficient of variation, ranged between 3%-7%, with the exception of one animal with a 10% CV. Maximum BFD (mean $\pm$ SEM) at day 1 was 4.48 $\pm$ 0.33 cm (range 3.10- 5.70) and a tendency to decline over time

was marginally significant (P = 0.055) (Fig. 2). Mean body mass declined significantly (P < 0.001) (Fig. 3), but BCS showed a trend to increase (P = 0.13) (Fig. 4). Mean BRI did not change during the 14d period (P > 0.05) (Fig. 5). IFBF estimated from BRI was a significantly related to MBF (adj. $r^2 = 0.72$; P = 0.002) (Fig. 6). Maximum BFD was more closely correlated with BM and BRI than with BCS (Table 1).

Discussion

The accuracy in measurement of BFD with this technique is 0.1 cm and is within the accuracy needed to detect significant physiological changes in a live animal. The CV measured for each animal in this study is high because there was a tendency for each animal to decline in maximum BFD over the 14-d study period. In these reindeer, ultrasonic rump fat thickness accounted for 81% of between animal variations in body mass on day 1. The trend for subcutaneous back fat to decrease over time probably accounts for the slightly higher than expected inter-assay variation (3-7 %). Observed echogenic shifts appear to be related to temperature changes within the tissue. Packing the rump region with snow for a period of 10 minutes resulted in an increased echogenicity of the fat layer and a homogenous appearance of both tissue types. The effect was reversible.

Efficacy of the technique is related to fat levels. The animals used in this study were moderately obese. Mean maximum BFD (4.48 cm) was similar to female Svalbard reindeer (*R.t. platyrynchus*) (4.48 $\pm$ 0.96 cm, Reimers & Ringberg, 1983) and exceeds that usually found for female caribou (*R.t. granti*) in autumn (0-3 cm) (Dauphine, 1976;

Chan-McLeod et al., 1995). In animals for which no backfat is detectable, results from reindeer (Reimers & Ringberg, 1983) and caribou (Chan-McLeod et al., 1995), suggests body fat levels below 2 kg and 6% respectively, and some other measure must be used to estimate body fat. Of these possible measures (Chan-McLeod et al., 1995) only the palpation technique used to estimate BCS and BRI holds immediate application. Estimating the depth of kidney fat by ultrasound could be possible, but requires evaluation. In this study it is not surprising that within and between individuals the maximum BFD was highly correlated with BM, since the variables that affect BM other than body fat, mainly alimentary fill, lean body mass and level of hydration, were largely held constant in this cohort of non-lactating and non-pregnant reindeer.

Differences noted between initial and final values (mean, min., and max. BFD) suggest declines in all variables except BCS (Table 2) Body fat levels estimated from published prediction equations were between 8 to 10 kg and a decline of between 0.2 and 1.1 kg over the 14-d period was indicated (Table 3). An estimate for caribou using the equation of Chan-McLeod et al. (1995) projects over 21 kg fat, however this equation was developed for very lean caribou and may not be applicable to these more obese reindeer. Thus the small decline in maximum BFD from 4.5 to 4.2 cm over 14-d results in a decline in body fat of 0.2 to 1.1 kg, depending on the prediction equation used (Table 3). The decline in body fat predicted from the equation of Chan-McLeod et al. (1995) at 1.3 kg was higher and possibly spurious. The observation that a similar trend in body fat is obtained using maximum BFD or BRI as predictors adds evidence that BFD, as measured by the ultrasound technique, is useful to assess body fat in reindeer and caribou

with measurable depth of backfat. Measurement of BFD in extremely thin reindeer and caribou however is hampered by the sequential patterns of fat deposition, namely late deposition of backfat and its early use in late winter (Dauphine, 1976; Adamczewski et al. 1987;Chan-McLeod et al., 1995).

BRI was superior to BCS as a predictor of maximum BFD in these reindeer suggesting that BCS is a measure of both fat and muscle cover. The opposing temporal trends in BFD (decline) and BCS (increase) suggest that these animals are either depositing protein or are reconstituting backfat and muscle fat in December.

In conclusion, our results suggest that ultrasonographic assessment of subcutaneous back fat in reindeer is feasible provided the animals have measurable fat layers. Under conditions where there is no measurable backfat layer we suggest the BRI can be used to determine body fat reserves (Gerhart et al., 1996). Thus the combined estimation of BFD, BCS and BM provide the tools to estimate body condition of live animals. Our observations suggest that monitoring of BFD combined with BCS and BM could be used in studies involving fat synthesis and use. Accuracy of back fat level measurement is to the nearest 0.1 cm and thus is precise enough to detect physiological changes in a live animal.

Current drawbacks of the technique for field application are the required shaving of the hair coat and maintaining a constant standing position of the animal. The latter requires development of a standardized method of restraint in the standing position i.e. in a portable crush. However, accurate back fat measurements have been obtained in immobilized recumbent moose (Stephenson et al., 1993), which suggests that

ultrasonographic fat measurements may not depend on a standing position of the animal, but the approach needs to be verified for reindeer.

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Fig.1 Ultrasonographic image of rump region in female reindeer. Shown is a longitudinal cross-section. The left of the screen is in the cranial and the right side is in caudal direction of the animal. The white narrow band at the top is the skin followed by a thick layer of subcutaneous fat. Fat thickness is increasing from left to right. A bright narrow band, the fascia separates fat and muscle. Maximum thickness for this animal was 5.5 cm.

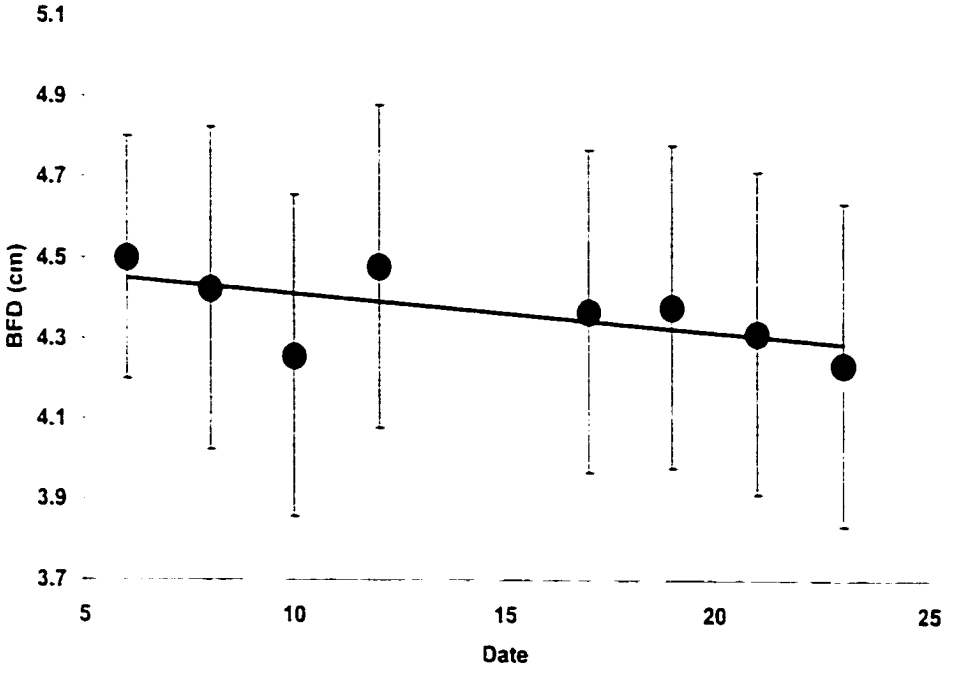

Fig. 2. Temporal profile of backfat depth (BFD) over a 2-week study period in adult non-pregnant reindeer, Alaska, December 1996. Data are expressed as mean ± SEM.

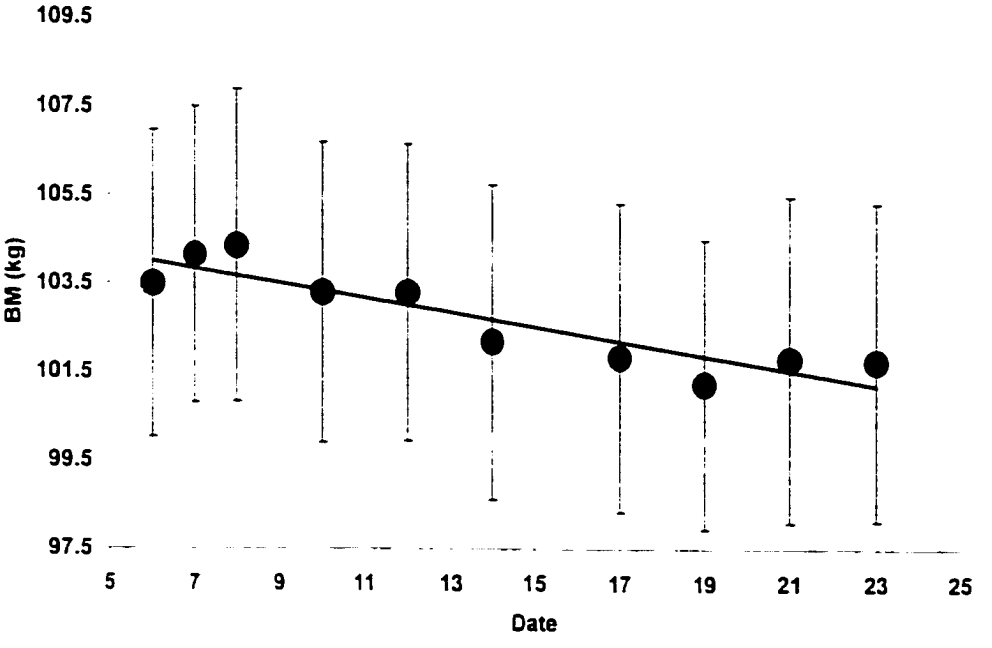

Fig. 3. Temporal profile of body mass (BM) over a 2- week study period in adult non-pregnant reindeer. Alaska. December 1996. Data are expressed as mean ± SEM.

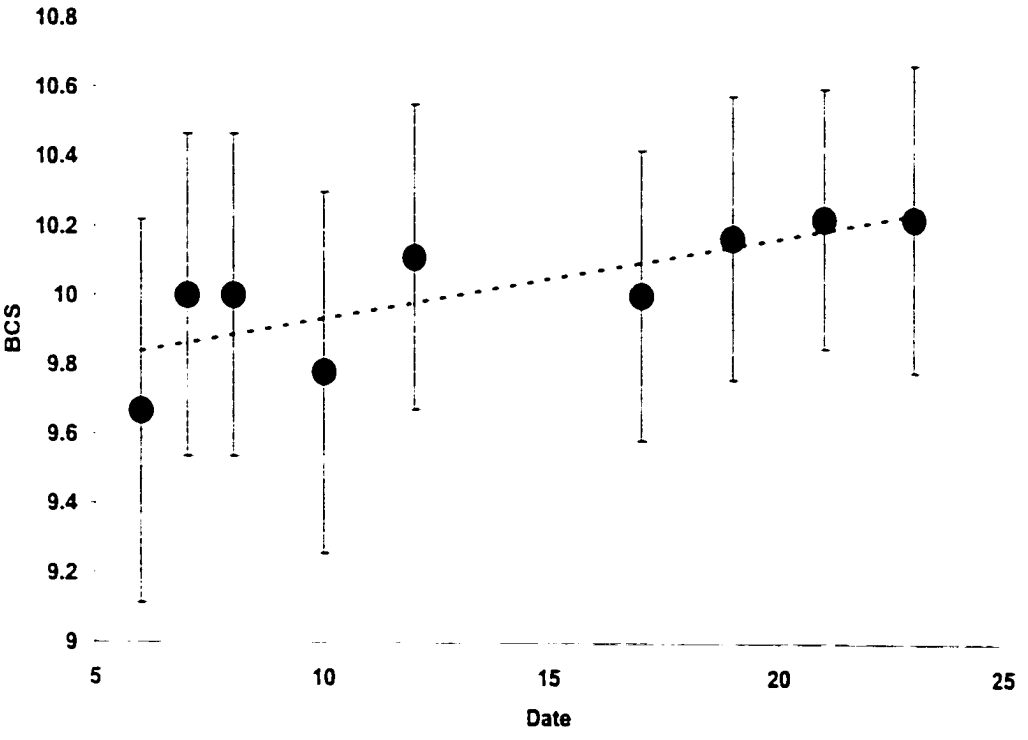

Fig. 4. Temporal profile of body condition score (BCS) over a 2-week study period in adult non- pregnant reindeer. Alaska. December 1996. Body condition score is defined in materials and methods section. Data are expressed as mean ± SEM.

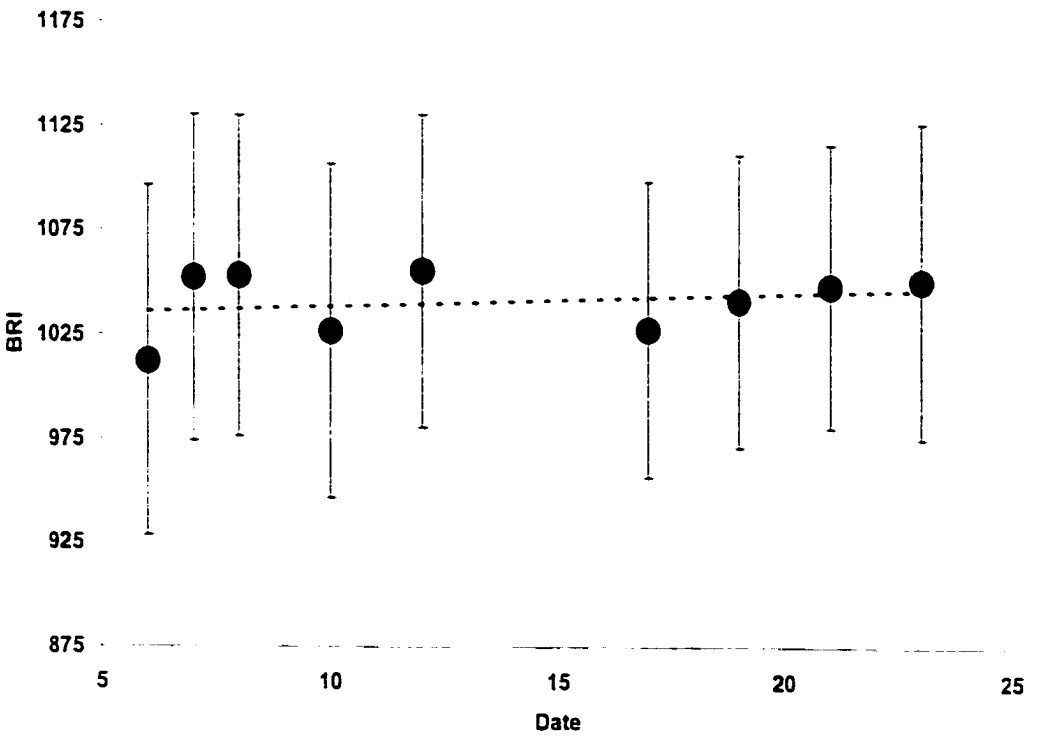

Fig. 5. Temporal profile of body reserve index (BRI) over a 2-week study period in adult non-pregnant reindeer. Alaska. December 1996. Body reserve index is given as BRI= BCS x BM. see materials and methods section. Data are expressed as mean ± SEM.

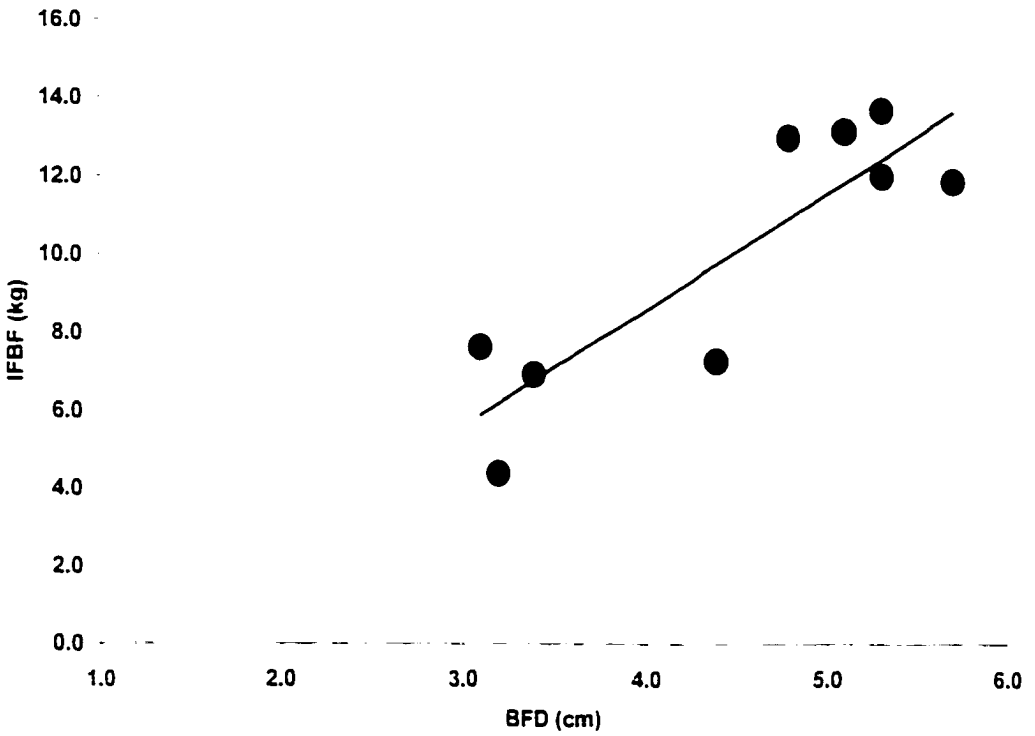

Fig. 6. Relationship between ingesta-free body fat (IFBF) and backfat depth (BFD) in adult non-pregnant reindeer in early winter. Ingesta- free body fat was estimated from a body condition score and body mass (Gerhart et al. 1996). see Materials and Methods. Data are expressed as mean ± SEM.

Table 1. Prediction of backfat depth (BFD) and body mass (BM) of female Alaskan reindeer, 1996. Body condition score (BCS) and body reserve index (BRI) are defined in Materials and Methods.

Variable	Equation	r^2	P-value
BFD (Y)			
BM (X)	Y = - 4.73 + 0.0865 •X	0.81	0.001
BCS (X)	Y = - 0.24 + 0.492 •X	0.61	0.022
BRI (X)	Y = 0.99 + 0.0034 •X	0.75	0.003
BM (Y)			
BCS (X)	Y = 55.6 - 4.9484 •X	0.62	0.01
BFD (X)	Y = 61.8 - 9.32 • X	0.81	0.001

Table 2. Initial and final values of body condition indices in adult non-pregnant reindeer, Alaska, 1996. Body mass (BM), dressed weight, body reserve index (BRI), body condition score (BCS) and backfat depth (BFD) are defined in Materials and Methods.

Time	BM (kg)	Dressed Weight [1] (kg)	BCS	BRI	BFD (cm)
START Day 1					
mean	103.2	57.8	9.6	1067	4.5
min.	89.1	49.9	6.5	579	3.1
Max.	115.9	64.9	11.0	1275	5.7
END Day 14					
mean	101	56.6	10.2	1050	4.2
min.	85	47.6	7.5	637	2.6
max.	112	62.7	11.0	1235	5.3
Difference in means	-2.2	-1.2	+ 0.6	-17	-0.4

[1]Ringberg et al., 1980

Table 3. Initial and final body fat estimated in adult non-pregnant reindeer. For equation and definitions of ingesta-free body fat (IFBF) see Materials and Methods.

	Body Fat (%)	Body Fat (kg)	IFBF (kg)	Body Fat (kg)	Body Fat (kg)
Equation	2	2*BM	3	4	5.1
START					
Day 1					
mean	20.5	21.1	10.5	7.8	10.0
min.	16.2	14.4	4.4	5.6	5.7
Max.	24.5	28.4	13.6	9.8	14.6
END					
Day 14					
mean	19.5	19.8	10.3	7.4	8.9
min.	14.7	12.5	5.0	4.8	4.4
max.	23.1	25.9	13.0	9.1	12.9
Difference in means	-1.0	-1.3	-0.2	-0.1	-1.1

[1] Ringberg et al., 1980
[2] Chan-McLeod et al., 1995
[3] Gerhart et al., 1996
[4] Adamczewski et al., 1987
[5] Reimers & Ringberg, 1983

Chapter 2

Meal patterns of reindeer (*Rangifer tarandus*) fed a concentrate diet in winter[1]

Abstract

Twenty-four hour feeding activity was recorded in reindeer (*Rangifer tarandus tarandus*) fed a concentrate diet under natural photoperiod conditions during early winter (16 Dec-3 Jan) in Fairbanks Alaska (64° 49' N, 147° 43' W). Distinct diurnal and nocturnal feeding events, with significantly more feeding occurring during daytime, were recorded. An increase in feeding followed sunrise but not sunset. Mean daily dry matter intake (1.18 ± 0.08 kg) did not change significantly. Of 121 meal-intermeal cycles analyzed over 2 wk, daily number of meals (7.1 ± 0.3) and mean meal size (162 ± 10 g) were not randomly distributed. Meal size varied from 50-550 g with small meals (< 101 g) (41%) during daytime, and fewer and sometimes large meals (> 325 g) occurring at nighttime. However, daytime meal size (156 ± 10 g) was not significantly different (P=0.16) from meal size during nighttime (177 ± 19 g). Significant positive correlations were found for meal size on duration of pre-meal interval ($r^2 = 0.92$, $P = 0.042$) and post-meal interval on meal size ($r^2 = 0.79$, $P = 0.017$). A test of two models based on either incurred energy deficit since time of last meal (metabolic model) or meal size equal to deficit in rumen dry matter fill (physical model) could not explain the large nighttime meals, although the metabolic model best fitted the daytime meal patterns. Separate analysis of diurnal and

[1] Prepared to be submitted to Can. J. Zool. as Stimmelmayr, R. & White, R.G. Meal patterns of reindeer (*Rangifer tarandus*) fed a concentrate diet in winter.

nocturnal meals indicated that the nocturnal meal pattern was better explained by the physical model.

Introduction

Voluntary food intake (VFI) varies seasonally in many north temperate ungulates. For example in *Rangifer* daily dry matter intake (DDMI) is down regulated in winter (McEwan and Whitehead 1970; White et al. 1984; Larsen et al. 1985). DDMI is a function of the number and size of meals each day and the respective inter-meal intervals. Daily temporal organization of meals results in characteristic meal patterns. Hormones, food availability, food quality and animal-animal interactions can modify daily meal patterns. Daily meal patterns also show rhythmicity, which varies depending on whether the species is mainly nychthemeral (poly-cyclic across 24 h), twilight active (crepsicular), active at sunrise and sunset (crepuscular), and diurnal and/or nocturnal in feeding behavior (Errikson et al. 1981; Sibbald 1994; Maier and White 1998; Colman 2000). Much behavioral activity is modulated by the daily photoperiod, which is transduced to the organism via amplitude and duration of nocturnal secretion of the pineal hormone melatonin (Loudon 1994). An important aspect of VFI by north temperate ruminants in winter is the apparent relatively tight linkage between energy intake and energy requirements, such that under *ad libitum* feeding, body mass shows only a modest decline throughout winter.

Few wildlife studies have measured individual meal size in relation to interval between meals. Food intake studies with arctic wildlife have emphasized logistic

relations between eating rate and food handling time in relation to food structure and availability (White and Trudell 1980; Trudell and White 1981). Much of the theory on the interrelation between meal size and inter-meal interval derives from studies with mostly diurnally active domestic ruminants. In sheep fed high quality diets (chopped hay-concentrate of >65% apparent dry matter digestibility) at a level where body mass remains constant (i.e. maintenance) meal size up to 300 g is correlated with the pre-meal interval. but the post-meal interval is not correlated with the meal size (Baile 1975). In the ruminant a tight linkage of meal size and pre-meal interval is an indicator that energy needs are met by intake (i.e. metabolic control). Thus Baile's data for concentrate diets support some form of metabolic control over meal size rather than a constraint imposed by the rate of processing and passage of food particles as shown for roughage diets (i.e. rumen deficit) (Campling 1970). A good test that meal size is dependent on the pre-meal interval would be to repeat this work in a species that exhibits both diurnal and nocturnal feeding activity and that has a strong endogenously regulated daily food intake.

We monitored 24 hour feeding activity of reindeer to determine daily meal patterns and DDMI during the winter solstice and to identify whether pre-meal interval or post-meal interval have the strongest correlations with meal patterns. The study was approved by the Institutional Animal Use and Care Committee at UAF.

Material and methods

Animals and measurements

Animals: Eight adult non-pregnant female reindeer (>1.5 years) were used for feeding behavior studies during winter 1996-1997 (16 Dec.- 3 Jan.). Animals were held at the Large Animal Research Station (LARS), Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks (64° 49' N, 147° 43' W) and had free access to a standard pelleted ration (UAF-RR, Alaska Garden and Pet Supply, Anchorage, AK 99510), which was 18-20% crude protein, 28 % neutral detergent fiber, 4-5% acid detergent fiber, and > 80 % in *vitro* dry matter digestibility. Snow was freely available. Maximum and minimum daily temperatures were recorded at the weather station of the Agricultural Forestry Experiment Station, Fairbanks, which is approximately 2 km from LARS. Animals used in this study were exposed to regular handling prior to the experiment to minimize stress. Reindeer body mass was measured daily (nearest 0.5 kg). Trends in body mass over time were tested with RMANOVA (SAS System Version 8).

Feeding behavior

Daily dry matter intake: All animals were held as a mixed group and fed communally. Each day at 09:00 -11:00 two individuals were placed in two adjacent pens and were fed individually. Daily food intake was determined as food offered minus refusals (measured to nearest 1 g) and daily food intake was corrected for dry matter content of fresh food by drying a sample to 100 °C in a convection oven for 24 h (Suttie et al. 1991). Because of the known effect of ambient temperature on DDMI (Kennedy et al. 1986), we adjusted

the observed DDMI to that at the mean ambient temperature (-18°C, 255 °K) using the equation of Blanchard (1983):

[1] $$Y = 3450 - 7.99 \bullet X$$

where Y = DDMI (g) and X = mean daily temperature (°K). DDMI and temperature-adjusted DDMI were compared with a Student's t-test (SAS System Version 8). We used the *F* statistic that SAS provides to determine whether variances were equal. When variances were unequal, we used the approximate *t*-statistic for unequal variances provided by SAS and Satterthwaite 's (1946) approximation for degrees of freedom. To test for temporal trends in DDMI and temperature-adjusted DDMI we used one-way RMANOVA (SAS System Version 8). To test for cyclicity of observed and temperature-adjusted DDMI over the study period a sine curve was fitted to the residuals of the regression analysis (Sigma Plot Version 5).

Individual meal size and inter-meal interval: Each single feeding pen was equipped with a feed bin attached to a weighing plate of an electronic balance (Model 610T, Arlyn Scales, Lynbrook, NY). The voltage output of each balance was continuously monitored every 5 min throughout the 24-h period, and data were stored automatically onto a computer. A program was written to determine time of onset and completion of each eating event, food eaten (g DM) and the inter-meal interval. Data were accepted only if the apparatus recorded the start and endpoint of a feeding event.

Daily feeding activity cycles and photoperiod

Hourly distribution of meals (meals/h) and mean meal size per hour (g/h) were calculated from original data sets from 24-h feeding activity over 15 d for 8 reindeer. To test the hypothesis that meals throughout the 24-h feeding period were equally distributed we compared the meal number/h of daylight (including twilight period prior to sunrise and following sunset) and the meal number/h darkness (time period of no daylight) using Student's t-test (SAS System Version 8). Daytime (including twilight period) and nighttime (total darkness) mean meal sizes were compared using Student's t-test (SAS System Version 8). We analyzed the 24-h data for daily rhythmicity. Rhythms were defined as (i) cycles with clear distinction between night active (nocturnal) and day active (diurnal), (ii) twilight active (crepsicular), (iii) active at sunrise and sunset (crepuscular), (iv) multiple oscillating or polycyclic system with activity peaks evenly distributed (nychthemeral). To test for type of cyclicity of daily feeding activity a sine curve was fitted to (i) the residuals of daily distribution of number of meals/h data set, and (ii) the residuals of daily distribution of mean meal size/h data set (Sigma Plot Version 5). We made the assumption: if reindeer were timing the beginning of feeding activity to sunrise, no or very little feeding activity should be observed prior to sunrise, and an increase in feeding activity should occur at or near sunrise. If sunset terminates daily feeding activity, a decrease in feeding activity at or near sunset should occur. To test for the effect of sunrise and sunset as *zeitgeber* for feeding activity we compared number of meals for the 3 h period prior and post-sunrise as well as for the 3 h period prior- and post-sunset using Student's t-test (SAS System Version 8).

Meal Pattern Analysis

To test the hypothesis that individual meal size is controlled by the energy deficit incurred since the last meal (Baile, 1975) data sets from 24-h feeding activity over 15 d for 8 reindeer were calculated as: number of meals/d, meal size distribution (g) 50-100, 101-175, 176-250, 251-325, 326-400, 401-475, 476-550, inter-meal interval distribution in 30 min increments (0-570 min), and the inter-meal interval distribution in 7 meal-size intervals (min. pre- and post-meal interval). A minimum cut-off of 50 g was used as the criterion for a meal (Baile 1975). Amounts of food consumed of less than 50 g were considered "nibbling" events. Baile's metabolic model is based on the typical diurnal feeding activity of domestic sheep. To account for diurnal and nocturnal feeding activity we separated meal events by daytime and nighttime.

We tested an alternate hypothesis that the rate of processing of food controls meal size and time between feeding events (Campling 1970). This model implies that meal size equals a deficit in rumen fill. Thus we related the post-meal interval to meal size using the same data set as we used to test Baile's hypothesis. Mean meal size for each size class was related to the mean intermeal interval (pre and post) associated with the size classes (Baile 1975). Relations between variables of the Baile and Campling hypotheses were determined with linear regression analysis. The regression equations and correlation coefficients represent only the mean responses for the group of reindeer. All regressions are reported with the significance of the model. Where the intercept was not significant the regression was forced through zero to test for sensitivity of the response (i.e. slope).

The test of Baile's hypothesis was that meal size should increase with increasing pre-meal interval; the test of Campling's hypothesis was that larger meals should result in longer post-meal intervals.

Estimation of energy deficits and rumen-fill

We used regression analysis to test the hypotheses that individual meal size is a function of an incurred energy deficit since the last meal (sensu Baile) or alternately, that meal size is a function of a deficit in rumen fill (sensu Campling). A theoretical energy deficit (ED) was calculated from time since the previous meal, and a theoretical deficit in rumen-fill (RD) was calculated from time since the previous meal. Mean responses of ED and RD and meal MEI ($kJ/kg^{0.75}$), respectively meals (g) within each meal size class were calculated for the group of reindeer. Relations between variables of the Baile and Campling hypotheses were determined with linear regression analysis. The regression equations and correlation coefficients represent only the mean responses for the group of reindeer. All regressions are reported with the significance of the model. Where the intercept was not significant the regression was forced through zero to test for sensitivity of the response (i.e. slope).

Energy Deficit: To determine the energy deficit incurred since the last feeding the exponential decline in metabolic rate following feeding was estimated using the response of Fancy (1986) for caribou, a subspecies of *Rangifer tarandus*:

[2] $$Y = 2108 - 224.78 \bullet \mathrm{Ln}(X)$$

where Y = metabolic rate (kJ/kg $^{0.75}$ • d), Ln = natural log and X = time since previous feeding event (d). The energy deficit at any time, t (d) (ED_t, kJ/kg $^{0.75}$) was calculated as metabolic rate during feeding (2108 kJ/kg $^{0.75}$ • d) minus the energy expended since the last meal:

[3] $$ED_t = 2108 - \int_0^t Y_{[2]} \bullet t$$

where $Y_{[2]}$ = estimate from eq. [2] and t = time since previous meal (min).

Metabolizable energy ingested in each meal (MEI, kJ/kg $^{0.75}$) was calculated as:

[4] MEI = meal DMI (g) * dry matter digestibility (0.91) * metabolizability of digested energy (0.82) (ARC 1980) * energy content of food (17.3 kJ/g)

Deficit in rumen-fill: The rumen deficit was estimated as rumen capacity minus the rumen fill at the time of feeding. Rumen capacity (RC, kg DM) was calculated from alimentary tract data for concentrate-fed reindeer during winter (Staaland et al. 1979; Staaland et al. 1983; Staaland et al. 1986; White et al. 1984). For a 42 kg reindeer RC (kg DM) was given by:

[5] RC = total alimentary wet mass (8.1 kg) * 0.80 (ratio of rumen content to total alimentary tract content) * 0.14 (dry matter fraction) = 0.907 kg DM

Since body mass and RC are linearly related in ruminants (van Soest 1981), we estimated RC as 0.907 * 1000/ 42 or 21.6 g DM / kg BM.

Rumen dry matter fill (RF_t kg/ kg BM) was assumed to decline exponentially with time since the last meal (t, d):

[6] $$RF_t = RC \bullet e^{-kt}$$

where k = rate-constant for rumen DM outflow (/d) and t = time since feeding; k was calculate as the inverse of rumen DM turnover time, which is related to DDMI via:

[7] $$Y = 35.0 - 0.394 \bullet X$$

where Y = rumen turnover time (h) and X = DDMI ($g/kg^{0.75}$) (White et al. 1984). Following feeding the difference between RC and RF_t was defined as the rumen deficit (RD_t, kg DM):

[8] $$RD_t = RC - RF_t$$

In this model we assume that hunger, caused by low RF brings about a feeding event that fills the rumen to capacity. Thus meal size should be statistically equal to the RD created by the pre-meal interval, or that the regression of meal size on RD is statistically significant, but without a significant intercept. [We built two models to evaluate how closely individual animal data sets agree with meal size and feeding frequency in relation to an energy deficit incurred since the last meal (metabolic model), and meal size and feeding frequency in relation to food rate of passage estimated as a deficit in rumen fill since the previous meal (physical model). Appendix 1]

Results

Photoperiod and Temperature

Mean hours of possible total daylight (sunrise to sunset) were 3 h 51 min ± 30 min. Photoperiod during our study period declined to 3 h 43 min (21 Dec), stabilized (22-25 Dec), and thereafter increased to 4 h 12 min (Fig.7). Mean temperature was -13.6

$\pm$ 2.5 ^{O}C and temperature declined over the study period with a maximum of 9 ^{O}C (17 Dec) and a minimum of –43.5 ^{O}C (3 Jan).

Body mass

Reindeer body mass ranged between 84.5 and 114 kg. Mean group body mass was 104 kg. There was no significant trend with time in daily mean body mass over the study period (RMANOVA, $F_{[7,13]} = 0.37$, $P = 0.98$). Between- animal variability in mean mass was significant ($F_{[7,13]} = 445.6$, $P = 0.0001$) (Fig.8).

Feeding behavior

Animals adapted well to the individual pens and ate regularly within the individual pen. Nibbling was not observed. Seventeen 24 h feeding activity data sets were recorded during the study period. A typical feeding pattern with feeding events intermittently spread throughout the day is shown in Figure 9. Total number of meals analyzed was 121. Mean DDMI was 1.18 $\pm$ 0.08 kg with a minimum of 0.60 kg and a maximum of 1.89 kg. Temperature-adjusted mean DDMI was 1.04 $\pm$ 0.08 kg with a minimum of 0.52 kg and a maximum of 1.71 kg. Temperature adjusted DDMI was not statistically different from DDMI (T-test, df = 42; $P = 0.296$). There was no significant overall temporal trend in DDMI (RMANOVA, $F_{[11,11]} = 0.81$, $P = 0.634$) and temperature-adjusted DDMI (RMANOVA, $F_{[11,11]} = 0.88$, $P = 0.58$) over the time period (Fig. 10). Test for cyclicity was not significant for DDMI (Regression, $F_{[2,21]} = 2.05$, $r^2 =$

0.16. P = 0.154) and temperature-adjusted DDMI (Regression, $F_{[2,21]}$ =3 .09, r^2 = 0.23. P = 0.067).

Feeding cyclicity and timing to sunrise and sunset

Daily feeding pattern for the study period was characterized by 2 main peaks of feeding activity, one during daytime (11:00 -16:00) and one broad peak at nighttime (22:00 - 05:00) (Fig. 11). The beginning of daytime activity occurred around the time of dawn (study period: *dawn* 10:51-10:56; *dusk* 14:37-14:52) and it declined around dusk. Nighttime activity commenced after a 1 h rest and extended to an abrupt end at 05:00 approx. 4-5 h before dawn and the beginning of daytime activity. Two daytime peaks, a major peak at 11:00 followed by a second peak between 16:00 and 19:00, characterized daily distribution of mean meal size through the 24h. Two peaks: one at 19:00 and the other characterized the nighttime distribution of mean meal size at time of day at 24:00. Only 3 meals contribute to the major daytime peak (11:00) with one inordinately large meal (550 g) appearing to be driving the peak. Without the single large meal, mean meal size at 11:00 was 175 g and thus less than the mean meal size of 225 g at 12:00. Reindeer ate more meals/h during daytime (1.0 $\pm$ 0.26/h) than nighttime (0.33 $\pm$ 0.05/h) (T-test, df = 8; P = 0.017). Mean nighttime meal size was 177 $\pm$ 19 g and was not significantly different from mean daytime meal size of 156 $\pm$ 10 g (T-test; df = 71; P = 0.158). Analysis of daily distribution of number of meals/h indicated diurnal and nocturnal feeding activity extended each for approximately 12 h with troughs between diurnal and

nocturnal feeding activity at 09:00 and 21:00 (Regression, $F_{[2,21]} = 12.18$, $r^2 = 0.54$, $SE_{estimate} = 3.025$, $P = 0.0003$).

[9] $$Y = 4.34 \bullet \sin(\Pi \bullet (X - (-11.77))/5.89)$$

where Y = residuals number of meals/h and X = time of day. Daily distribution of meal size/h indicated a polycyclic rhythmicity with a 7 h periodicity (Regression, $F_{[2,21]} = 3.59$, $r^2 = 0.26$, $SE_{estimate} = 59.67$, $P = 0.046$).

[10] $$Y = 47.097 \bullet \sin(\Pi \bullet (X - (-7.0212))/4.23)$$

Where Y = residuals for number of meal size / h and X = time of day (h). Mean number of meals in the 3 h period prior to sunrise (0.33 ± 0.33) was lower than in the 3 h period post sunrise (7.33 ± 1.89) (T-test, df = 2, P = 0.033). Mean number of meals in the 3 h period prior to sunset (7.33 ± 1.86) was not significantly different from that in the 3 h period post sunset (9.67 ± 3.38). (T-test, df = 4, P = 0.29).

Meal Pattern Analysis

Number of meals/ d: Mean number of meals per day was 7.1 ± 0.3 with a range of 5-10. Mean meal size was 162 ± 10 g with a range of (50-550).

Number of meals in 7 meal-size intervals: Reindeer consumed meals of between 50 and 550g (Table 4). Small meals dominated with 41.3 % of meals less than 101g, followed by 19.8 % for meals > 101 < 175 g, 25.6 % for meals > 176 < 250 g and 13.3 % for meals > 251 g. Daytime and nighttime composition of meal sizes indicates a mixed composition (70 % daytime: 30 % nighttime) for small and intermediate meals (< 250 g) while the majority (78 %) of the fewer large meals (>325 g) occur during nighttime (Fig. 12a).

Single large meals (> 250 g) occurred on 16 occasions over the study period, usually one each day, but on four occasions two large meals occurred per day. On five occasions no large meals were recorded. Percent of total meals (Y) and meal size (X g) were inversely related (Regression, $F_{[1,5]} = 22.06$, $r^2 = 0.82$; $SE_{estimate} = 7.16$, $P = 0.0054$).

[11] $$Y = 39.4 - 0.084 \bullet X$$

Inter-meal interval (mean, pre- and post-meal interval): Mean inter-meal interval was 150 ± 12 min with a range of 30 - 610 min with the majority of the inter-meal intervals were less than < 211 min (80 %) (Fig.12b). Pre and post-meal interval within 7 meal size intervals ranged between 125-465 min, and 149-180 min respectively (Table 4). Daytime (D) and nighttime (N) organization of meals within 7 meal size intervals for all pre-meal intervals were (i) 44 % vs. 56 % for 50-100 g; (ii) 28 % vs. 72 % for 101-175 g; (iii) 43 % vs. 57 for 176-250 g; (iv) 20 % vs. 80 % for 251-325 g; (v) 0 % vs. 100 % for 326-400 g; (vi) 0 % vs. 100 % for 476-550 g. Thus daily composition of meals within meal size intervals < 325 g was characterized by mixed composition (D and N), while meals within meal size intervals > 326 g were of uniform composition (N). Linear regression of mean meal size (Y g) on mean responses of pre-meal interval (X min)(Fig. 13a: line A) marginally reached significance with 64 % of the variation in meal size being explained (Regression, $F_{[1,4]} = 7.39$; $r^2 = 0.64$, $P = 0.053$). Without the two large nighttime meal size classes 92 % of the variation in meal size was explained (Fig 13a: line B) (Regression, $n = 4$, $F_{[1,2]} = 22.37$, $r^2 = 0.92$, $SE_{estimate} = 33.63$, $P = 0.042$).

[12a] $$Y = 2.93 \bullet X - 280$$

The intercept was not statistically significant (t =-2.82, P = 0.106) thus with the intercept forced through zero the slope was 1.21 (P = 0.008) and Se_b = 0.19.

[12b] $$Y = 1.21 \bullet X$$

A curvilinear regression model that included the two large nighttime meal size classes (Fig13a, line C) explained 73 % of the variation in meal size (Regression. $F_{[1,4]}$ = 10.54. r^2 = 0.73. $SE_{estimate}$ = 91.42. P = 0.031).

[12c] $$Y = 280.64 \bullet LnX - 1201$$

Where Ln is the natural logarithm. The curvilinear relationship appears to be driven by a single large nighttime feeding event.

Daytime (D) and nighttime (N) organization of meals within 7 meal size intervals for all post-meal intervals were (i) 40 % vs. 60 % for 50-100 g; (ii) 30 % vs. 70 % for 101-175 g; (iii) 30 % vs. 70 % for 176-250 g; (iv) 20 % vs. 80 % for 251-325 g; (v) 80 % vs. 20 % for 326-400 g; (vi) 100 % vs. 0 % for 401-475 g; (vii) 50 % vs. 50 % for 476-550 g. Thus daily composition of meals within all meal size classes was characterized by mixed composition (D and N), with the exception of meal size class 401-475 g, which was of uniform composition (D). Linear regression of mean responses of post-meal interval (Y min) on mean responses of meal size (X g) within each meal size class was not significant (Regression. $F_{[1,5]}$ = 1.93. r^2 = 0.28. $SE_{estimate}$ = 16.62. P = 0.223) (Fig. 13 b). Significance was achieved by removal of the single large daytime meal (401-475 g) (Fig13b. line D) (Regression. $F_{[1,4]}$ = 15.51. r^2 = 0.79, $SE_{estimate}$ = 8.61. P = 0.017).

[12d] $$Y = 131 + 0.09 \bullet X$$

DDMI (Y, kg) was not related to number of meals per day (X) (Regression, $F_{[1,9]} = 0.28$, $r^2 = 0.03$, $SE_{estimate} = 0.22$, $P = 0.61$).

Deficits in energy and rumen-fill

Energy deficit: Mean ED (62 ± 6 kJ/kg $^{0.75}$) created by the pre-meal interval was not statistically different from meal MEI (64 ± 4 kJ/kg $^{0.75}$) (T-test, df =163; P= 0.482). Linear regression of ED (X) on meal MEI (Y) (Fig. 14a) was not significant (Regression, $F_{[1,4]} = 5.98$, $r^2 = 0.60$, $SE_{estimate} = 40.47$, $P = 0.071$). Without the large nighttime meal MEI classes 95 % of the variation in meal MEI was explained (Fig. 14a, line A) (Regression, $F_{[1,2]} = 35.91$, $r^2 = 0.95$, $SE_{estimate} = 10.46$, $P = 0.027$).

[13a] $$Y = 2.6 \bullet X - 94.5$$

The intercept was not statistically significant (T =-3.31, P = 0.081) and with the regression forced through zero the slope was 1.17 (P = 0.006) with a $SE_b = 0.16$.

[13b] $$Y = 1.17 \bullet X$$

Although mean ED was statistically equal to meal MEI regression analysis suggests that meal MEI was 17 % larger than the deficit.

A curvilinear regression model that included both large nighttime meal MEI classes (Fig 14a, line B) explained 68 % of variation in meal MEI (Regression, $F_{[1,4]} = 8.46$, $r^2 = 0.68$, $SE_{estimate} = 36.22$, $P = 0.0438$).

[13c] $$Y = 87.6 \bullet Ln(X) - 278.3$$

Where Ln is the natural logarithm. The regression was driven by one large nighttime meal MEI class.

Deficit in rumen fill: Mean RD created by the pre-meal interval (0.26 ± 0.019 kg DM) was significantly larger than mean meal size (0.153 ± 0.009 kg DM) (T-test, df = 140, P < 0.001). Linear regression of meal size (Y) on RD (X) was significant and explained 73 % of variation in meal size (Fig 14b, line C) (Regression, $F_{[1,4]} = 10.69$, $r^2 = 0.73$, $SE_{estimate} = 0.09$, P = 0.0308).

[15a] $$Y = 0.065 + 0.5008 \bullet X$$

The intercept was not different from zero. With the regression forced through zero the slope was 0.62 (P = 0.0005) with a $SE_b = 0.08$.

[15b] $$Y = 0.62 \bullet X$$

With nighttime meal size classes removed (Fig 14b, line D) rumen deficit explained 99 % of the variation in meal size (Regression, $F_{[1,2]} = 1084$, $r^2 = 0.99$, $SE_{estimate} = 0.005$, P = 0.0009).

[15c] $$Y = 1.09 \bullet X - 0.135$$

Results of the modeling exercise (Appendix 1) supported the above results.

Discussion

In agreement with previous studies on reindeer, body mass of our animals remained relatively constant throughout the study period. Mean DDMI at 1.18 ± 0.08 kg (11 ± 0.7 g DM/ kg BM; 36 ± 2.3 g/ $kg^{0.75}$) is typical for reindeer in winter (McEwan and Whitehead 1970; Blanchard 1983, Ryg 1983; White et al. 1984; Mesteig et al. 2000) and combined with the finding of no significant trend in DDMI with date, ambient

temperature or of cyclicity, appetite appears well regulated. Reindeer in this study appear to have a VFI that approximates maintenance energy requirements.

Feeding behavior for these reindeer fed concentrate in winter was characterized by more meals occurring during daytime than nighttime. Activity patterns that suggest mainly diurnal feeding have been observed with captive Swedish reindeer (63° 49' N) in December and January (Erriksson et al. 1981) and Alaskan reindeer on the Seward Peninsula (Collins and Smith 1989). Erriksson et al. (1981) report two main periods of activity during daytime, one at dawn and the other at dusk. The duration of the mid-day resting period (13:00) was shorter in our study. This contrasts to a reported nychthemeral activity exhibited by reindeer grazing in the field at LARS (Maier and White 1998). Previous studies generally support timing of activity and feeding to sunrise in *Rangifer* (Roby 1978; Erriksson et al. 1981; Russell et al. 1993). However, field studies made with individual animals found no evidence (Maier and White 1998; Colman 2000). High variability among individual animals appears to contribute to the lack of a convincing trend among some of these studies. Thus it is likely that the different outcomes are partially due to methodological differences in data analysis, i.e. individual analysis versus group analysis and many instances a lack of 24-h observations biases the interpretation of data. In our study the test for timing of activity to sunrise was done on grouped data, similar to Erriksson et al. (1981). Our data did not support a role for sunset as a *zeitgeber*.

Although DDMI was representative of values for winter intake in concentrate fed reindeer (White et al. 1984; Larsen et al. 1985;) there was sufficient variation between days that shifts in meal size distribution were evident. The meal size distribution was

characterized by a preference for small meals (61.1 % of meals < 176 g), which resembled data for domestic sheep but a comparison of concentrate fed reindeer with sheep (Baile 1975) also shows that reindeer have more large meals, specifically those at night (Fig 15). Daytime and nighttime composition of meals indicates that 78 % of meals > 325 g occur at nighttime in reindeer. In sheep only 5 % of daily feeding activity occurs during nighttime (Colman 2000) and sheep do not normally eat large meals at night (Forbes 1980). Mean inter-meal interval (150 ± 12 min) was comparable with observations on inter-meal intervals from field studies where resting bouts in caribou during winter have a duration on average 126 ± 55 min during daytime bouts and 127 ± 73 min during nighttime bouts (Maier 1996). Comparison of relationships between meal size and inter-meal intervals show that the prediction of meal size from the pre-meal intervals was significantly better than the prediction of post-meal interval from meal size, and that meal MEI closely approximated the ED. The dependence of meal size on pre-meal interval is in agreement with Baile (1975) who showed that physical control of intake, as defined by Campling (1970), disappears when diet digestibility is greater than 65 %. The digestibility of the ration in this study exceeded 80 %.

The occurrence of large meals mostly occurred at night. This suggests a possible role for melatonin. When appetite is down regulated, as it is during winter in reindeer, a physiological "brake" is thought to control meals size (Bray 2000; Schwartz 2000). At night the brake appears released and it appears that the animal temporally overfills the rumen with an inordinately large meal (> 325 g). The meal is followed by a long post-meal interval when rumen contents are masticated, fermented and otherwise fully

digested and the number of nighttime feeding events is reduced. This scenario accounts for long post-meal intervals that follow a large meal, and it does not depend on a long pre-meal interval before occurrence of the first of the large nighttime meals. However, the theory does not hold for large daytime meals, which are preceded by a long pre-meal interval. The operation of a nocturnal blockade of an afferent signal on the appetite center could explain the large nighttime meal sizes. Corroborative data from laboratory rodents indicates a decreased post-prandial satiety during nighttime in response to afferent signals (Kraly et al. 1980). The involvement of melatonin as the factor permitting this change in appetite control deserves further study.

Conclusion

During early winter reindeer fed a concentrate diet displayed distinct diurnal and nocturnal feeding behavior with more meals occurring during daytime. Sunrise was a *zeitgeber* for feeding activity. Meal frequency and size distribution showed small frequent meals during the day and fewer and often larger meals at night. On a daily basis intake was constant as was body mass over the 2 wk study period. During daytime, meal MEI approximated an energy deficit incurred since the last meal, but large nighttime meals were not explained by this relationship. The occurrence of large meals drove the nocturnal meal pattern that was better explained by a deficit in rumen fill than by an energy deficit. In an environment that is characterized by several months of nearly 24 h darkness a combination of large nighttime meals and seasonally reduced metabolic rate may minimize negative energy balance throughout the winter and thus allow reindeer to

maintain fat reserves in an energetically challenging environment. Particularly for pregnant reindeer, sufficient energy stores must be maintained to drive late fetal protein deposition and to initiate lactation before adequate food is available.

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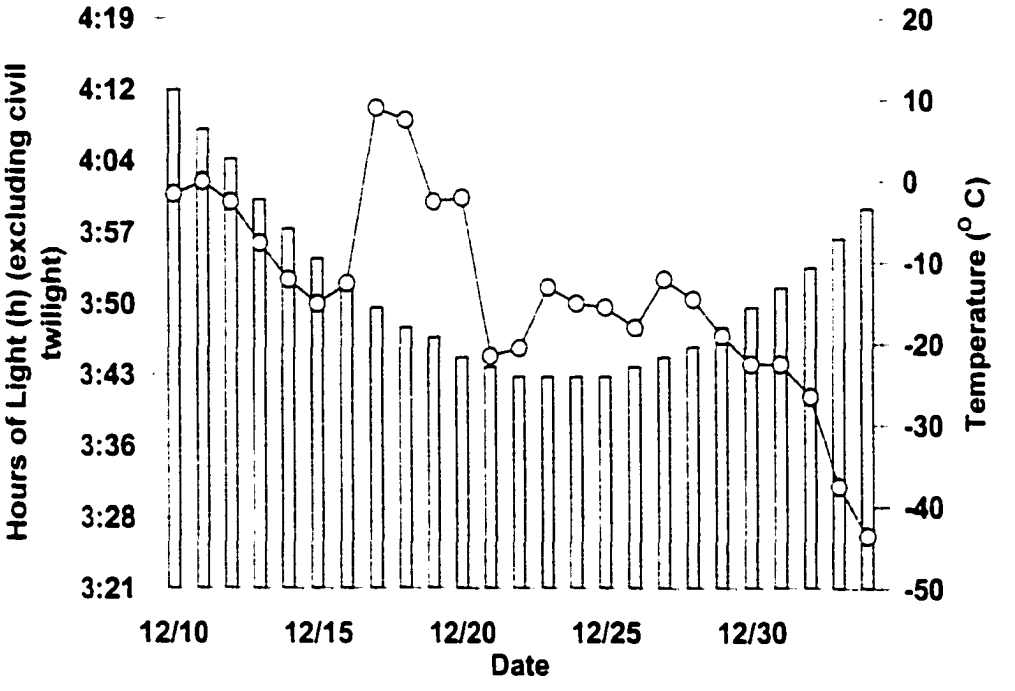

Fig. 7. Change in mean hours of total daylight (bars) and ambient temperature (o-o) during mid-winter at the Agricultural Experiment Station, University of Alaska Fairbanks, during the study period 1996-1997.

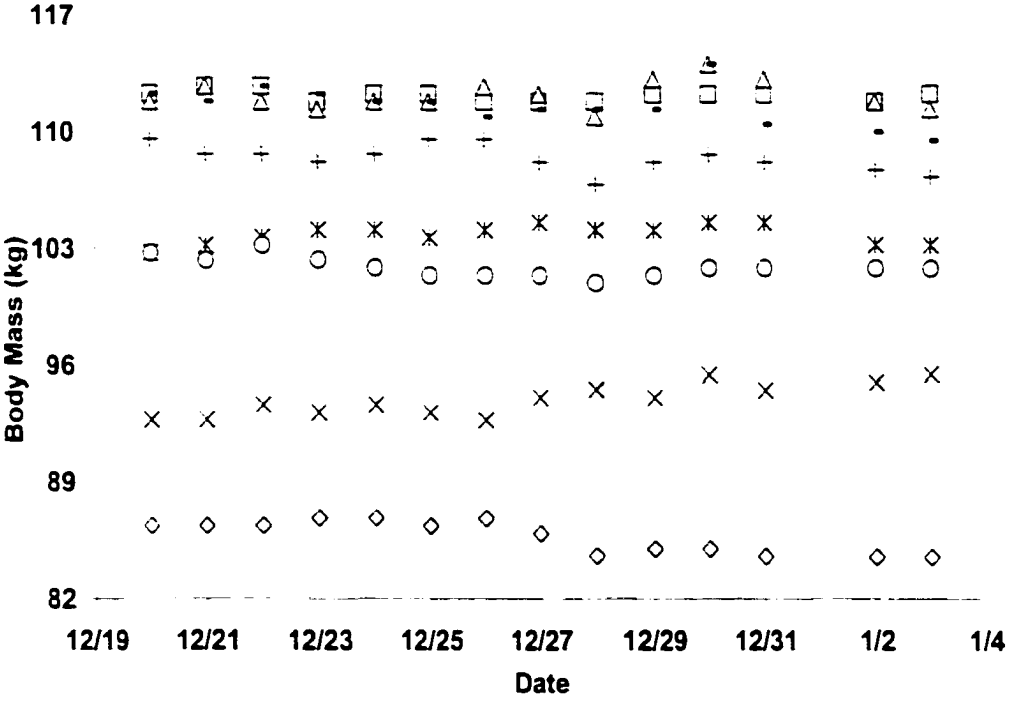

Fig. 8. Daily body mass of individual reindeer (n = 8) during mid-winter study period at LARS, Alaska 1996-1997. Each reindeer is given by a separate symbol and animals were weighed between 08:00 and 09:00 daily.

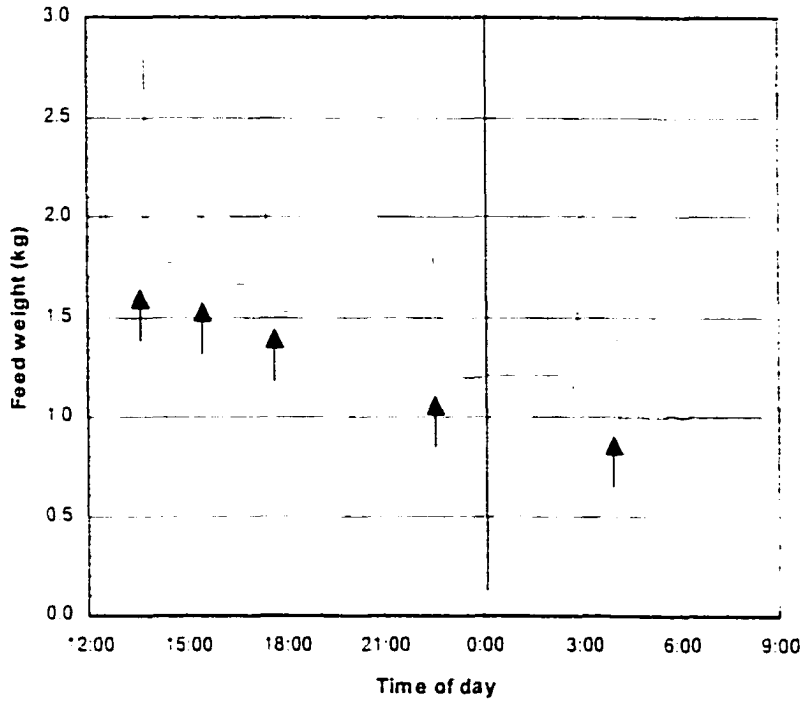

Fig. 9. Example of a daily trace in feed bin weight for a reindeer (12 Dec. 1996) at LARS, Alaska. The trace shows disturbances to the scale as the animal feeds and a steady line (inter-meal interval) between feeding events. Each meal was calculated as the decrease in feed weight following each feeding event. Inter-meal intervals were estimated as time between feeding events (arrows).

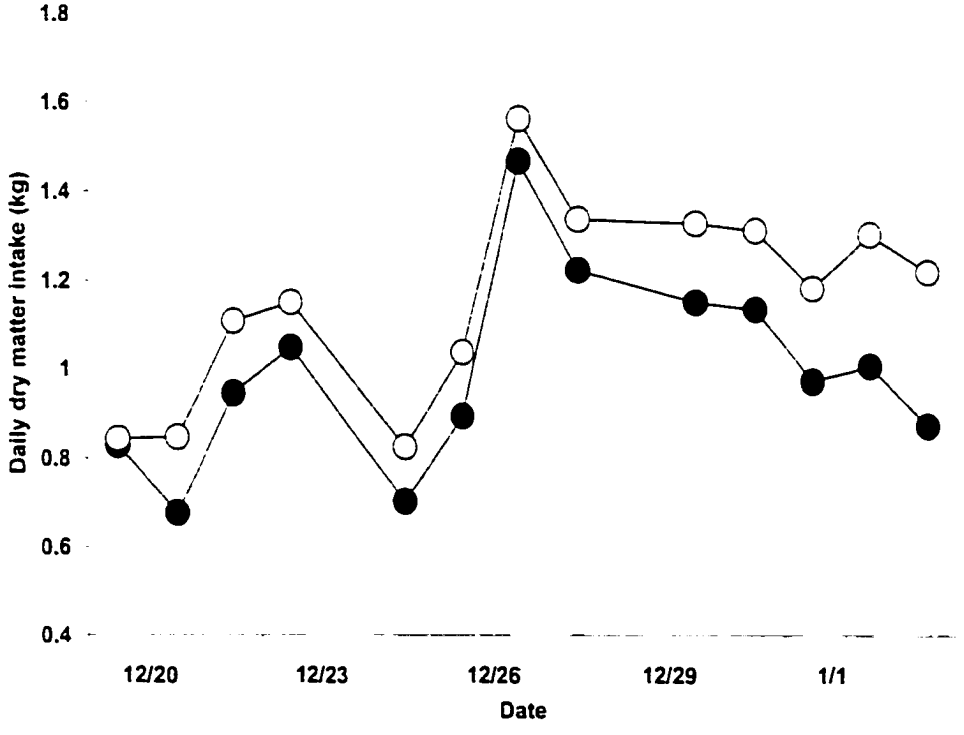

Fig. 10. Change in daily dry matter intake (o) and temperature adjusted intake (•) of 8 reindeer fed a concentrate ration at LARS, Alaska 1996-1997.

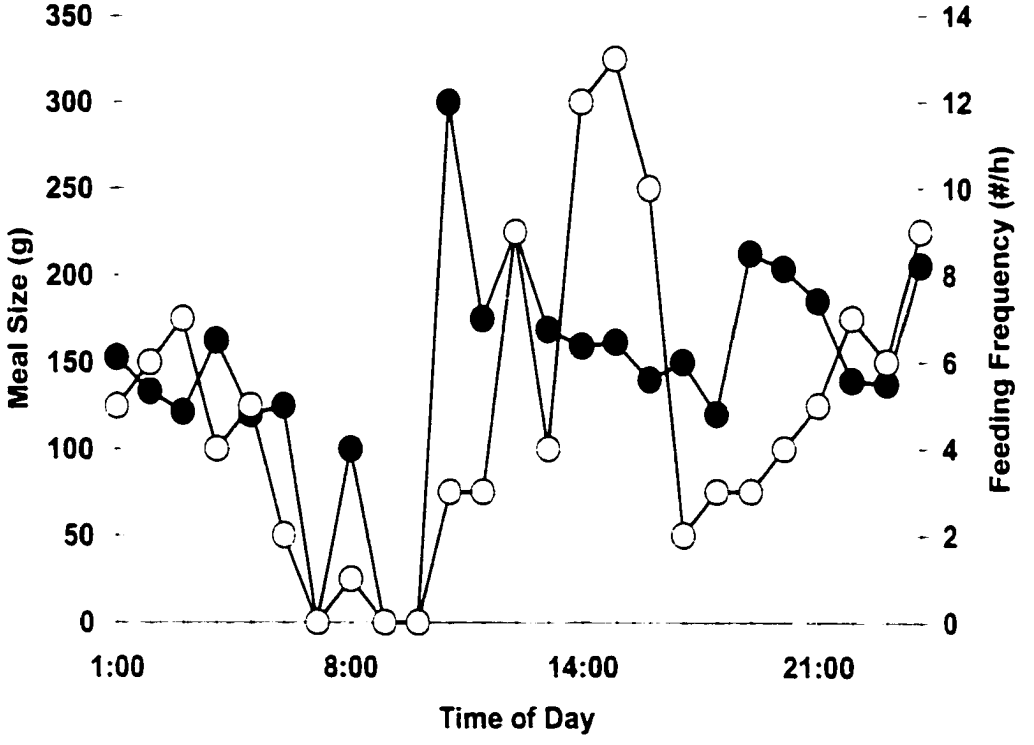

Fig.11 Meal size and frequency of feeding. Twenty-four hour distribution of mean meal size (•) and frequency of feeding (o) by concentrate fed reindeer at LARS. Alaska 1996-1997.

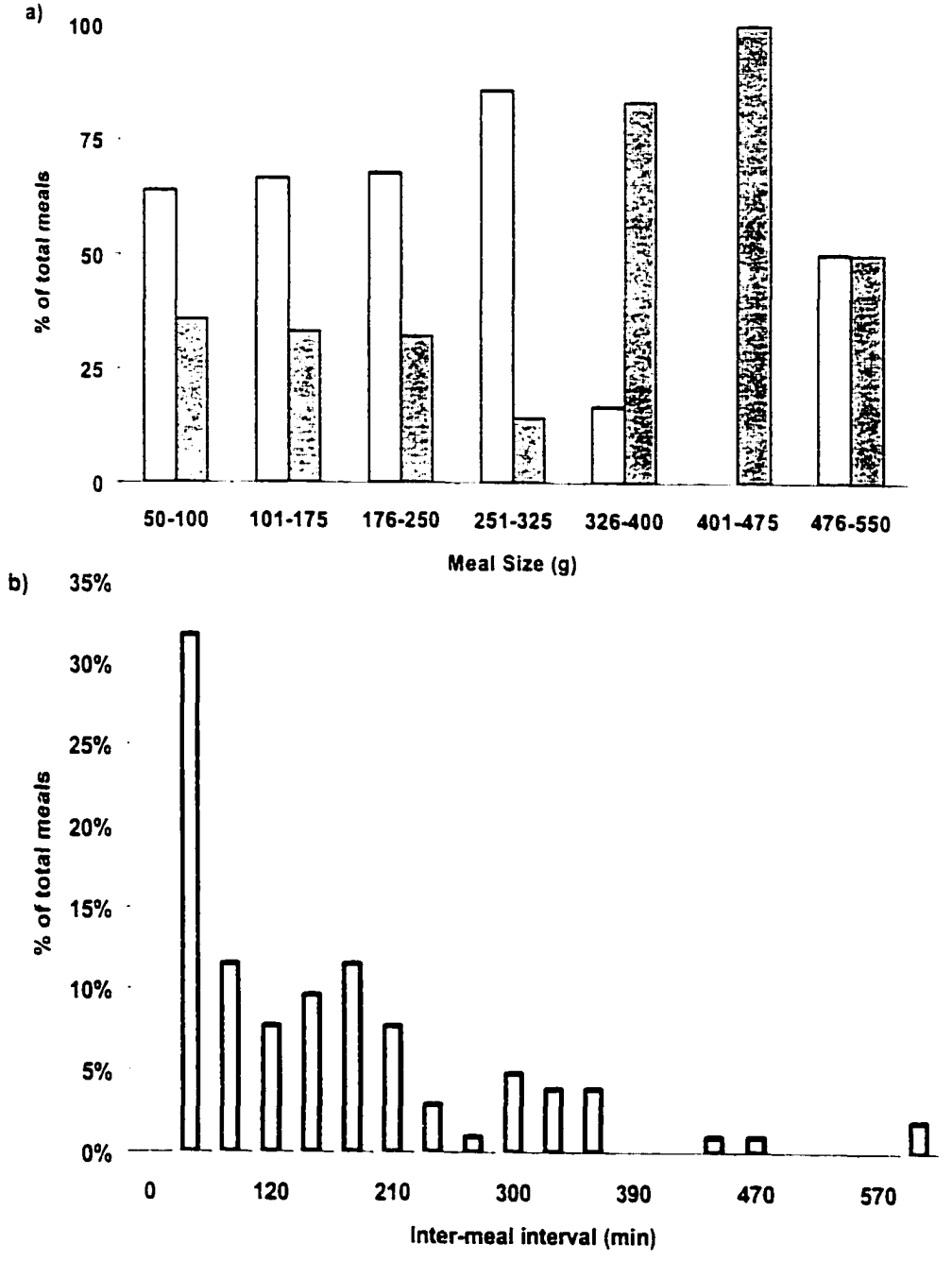

Fig. 12. Daytime (open bars) and nighttime (shaded bars) meal size distribution (a) and inter-meal interval distribution (b) for concentrate fed reindeer at LARS, Alaska 1996-1997. Total number of meals analyzed was 121.

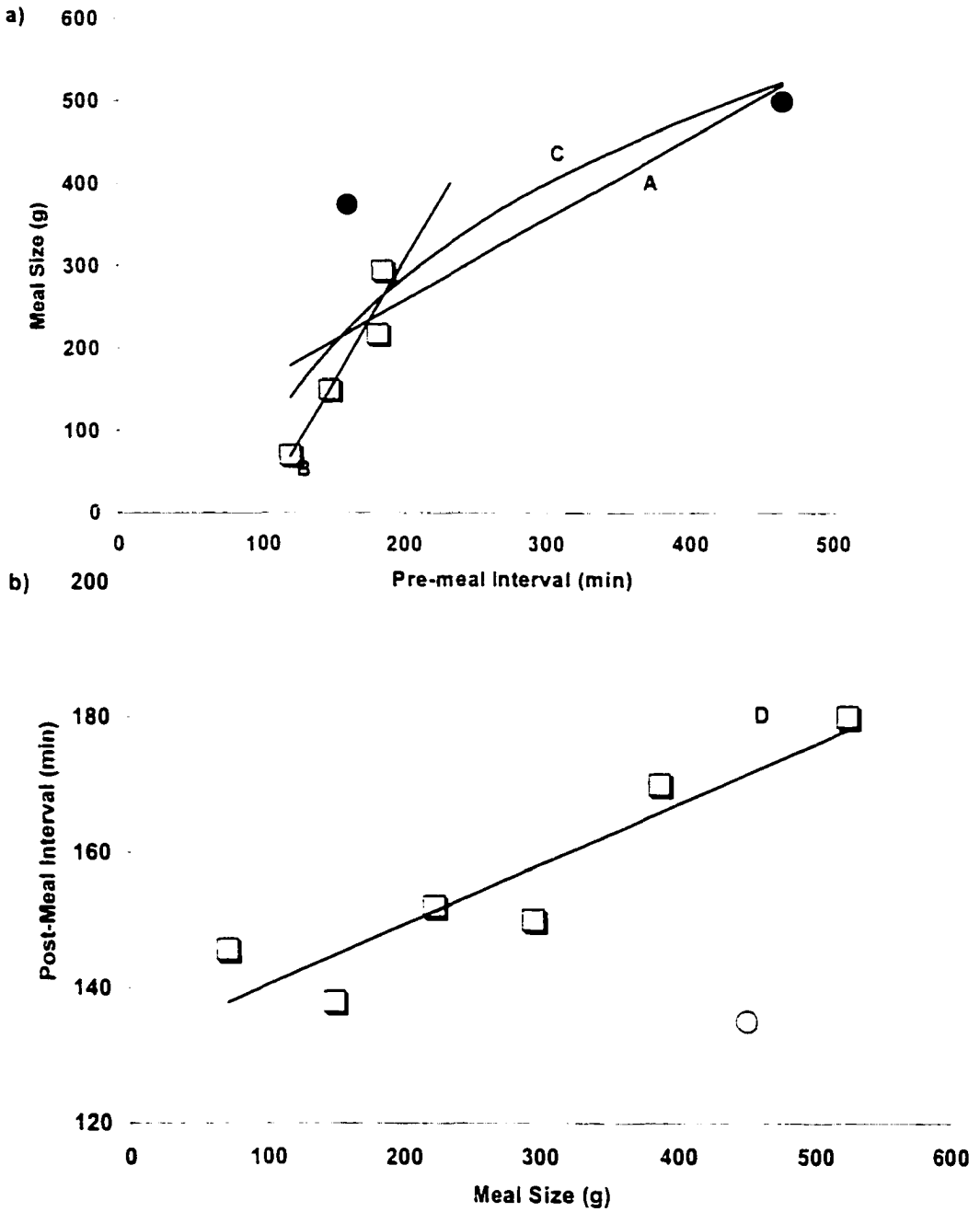

Fig.13. Relations between meal size and pre-meal interval (a) and between post-meal interval and meal size (b) for concentrate fed reindeer at LARS, Alaska 1996-1997. Daytime (o); nighttime (•); mixed composition (shaded squares). Line A is linear fit for all meal size classes; Line B is linear fit for the mixed composition meal size classes; Line C is a curvilinear fit for all meal size classes; Line D is linear fit for mixed composition meal size classes.

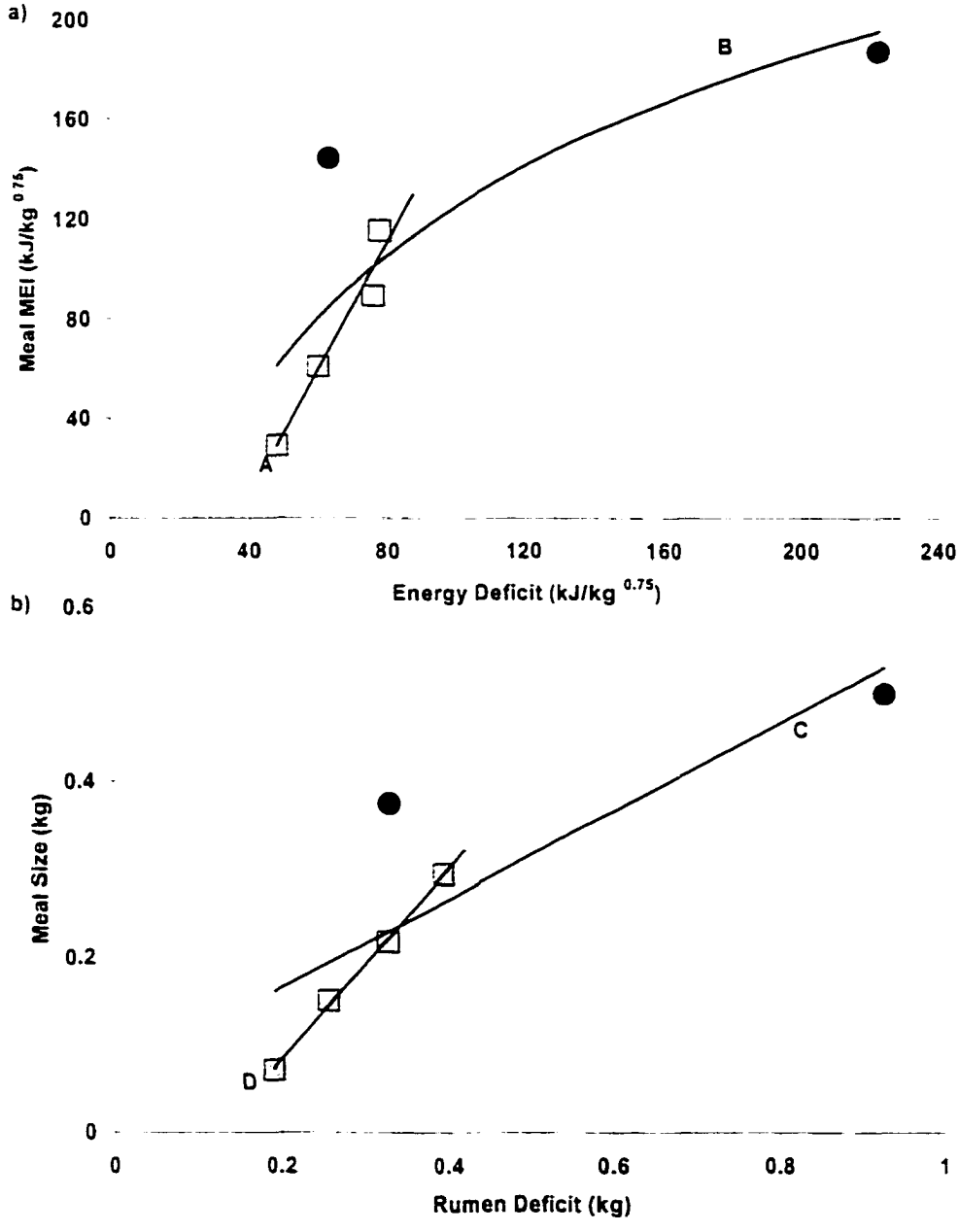

Fig. 14. Dependence of meal size on deficits in energy or rumen fill. Relationship of meal metabolizable energy intake (MEI) with energy deficit (a) and meal size with the deficit in rumen fill (b) in concentrate fed reindeer at LARS, Alaska 1996-1997. Daytime (o), Nighttime (•); Mixed composition (shaded squares). Line A is linear fit for mixed composition; Line B is curvilinear fit for all meal size classes. Line C is linear fit for all meal size classes; Line D is the linear fit for mixed composition meal size classes.

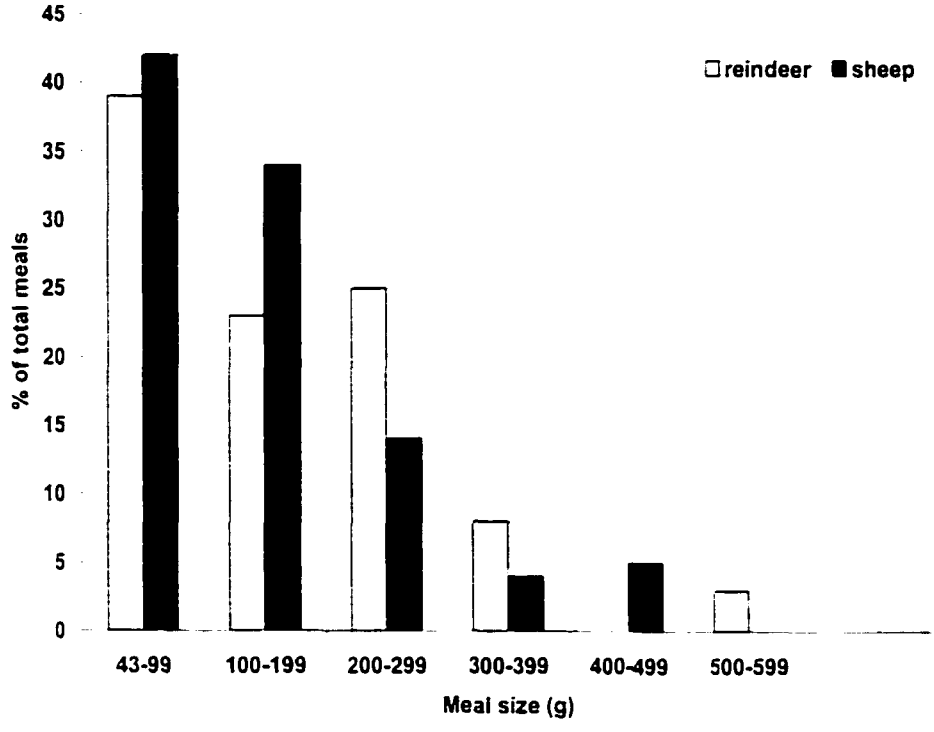

Fig. 15. Comparison of meal size distributions of reindeer (this study) with domestic sheep (Baile 1975).

Table 4. Meal analysis of feeding records for reindeer (n = 8) fed a concentrate ration at LARS, Alaska 1996-1997. Total number of meals analyzed was 121.

Variables	Meal Size Range						
	50-100	101-175	176-250	251-325	326-400	401-475	476-550
Percent of total meals	41.3	19.8	25.6	5.8	5.0	0.8	1.7
Meal size (g)	73±4	150±2	220±4	299±5	379±10	450	525±25
Pre-meal (min)	125±19	142±25	182±19	165±42	160±13		465
Post-meal (min)	149±19	138±28	152±24	150±61	138±16	135	180

Chapter 3

Serum insulin, glucose and lactate concentrations during 18 h fast in female reindeer[1]

Abstract

We investigated whether secretion of insulin occurred in the absence of feeding in a ruminant. Serum insulin, glucose and lactate concentrations were measured in three adult non-pregnant reindeer at hourly intervals during an 18 h fast (17:30-11:30 h) in October. Mean serum insulin concentration was 39 ± 3 μIU/ml (range 2-100 μIU/ml). The insulin profile of two animals was characterized by a bi-phasic cyclicity including a nocturnal rise and an early morning trough followed by an early midday peak. Within the peaks, minor peaks 2-3 h apart were noted. A sine curve fit to the residuals about the regression of insulin with time was significant and gave a period length of 9 and 6 h. For the third animal insulin secretion profile was characterized by short-term oscillations with 2-3 h periodicity. Serum glucose concentrations significantly increased during the fast in two reindeer, but were erratic in the third animal. Serum lactate concentrations declined significantly in all three animals. Sine curve analysis of serum glucose and lactate profiles indicated statistically significant 6-12 h periodicity of serum glucose concentration and 10-16 h periodicity of serum lactate concentration. Correlation of residuals for serum glucose concentrations were significantly negatively correlated to those of serum lactate, but were not related to serum insulin residuals. The 2-3 h oscillations of serum insulin

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secretion were of similar duration as the intermeal interval estimated for pen-fed reindeer during winter (2.5 h). Although not necessarily causal, the results are consistent with an hypothesized role for insulin in meal initiation.

Introduction

Insulin, a key hormone in the regulation of glucose homeostasis, food intake, nutrient storage and nutrient partitioning, exhibits pronounced seasonal fluctuations in reindeer characterized by a peak concentration in blood during summer and low levels during winter (Larsen et al. 1985). These concentrations correlate with seasonal changes in food intake, number of feeding activity bouts (Maier, 1996; Maier and White, 1998) and body weight gain in summer and maintenance of body mass and low appetite in winter (White et al., 1984). Whether insulin levels undergo a circannual rhythm thereby constituting a central pacemaker, or are acting as a messenger of some other rhythm, i.e. appetite center or physiological factors, is not known. Most research speculates that summer hyper-insulimia is a product of hyperphagia, however this hypothesis has not been tested in reindeer. Daily and ultradian (<24 h) fluctuations of plasma insulin that are not clearly related to feeding behavior have been previously reported in other species (humans, Boden et al., 1996; pigs, Thaela et al., 1995; 1998, quails, Tedford and Meier, 1993). These findings argue for the existence of an endogenous pacemaker. The present study was designed to examine serum insulin concentrations for periodicity during an 18 h fast and to determine temporal inter-relations of serum insulin levels with serum glucose and lactate concentrations. If insulin oscillations during fasting were clearly

linked to changes in glucose concentration, the results would support the hypothesis that insulin concentrations reflect feeding activity, in this instance brought about by rumination. However, if insulin and glucose oscillations were unlinked during fasting then the results would support the endogenous pacemaker hypothesis. The study was approved by the Institutional Animal Use and Care Committee at UAF.

Material and Methods

Animals and measurements

Three adult non-pregnant reindeer (L; M; W) were used for this study (October 5-6 and 13-14). Animals were halter trained and accustomed to handling and blood sampling. Mean body mass (BM) was 114 ± 6 kg with a range of between 103-121 kg. Animals were held outdoors under natural photoperiod conditions at the Large Animal Research Station (LARS), Fairbanks, Alaska (64° 52' 39" N, 147° 49' 29" W). Jugular catheters (Angiocath® 14 gauge; 5.14 ") were placed one day prior to blood sampling. At 08:30 the following day, animals were led into small indoor stalls. Size of the individual indoor stalls permitted animals to stand or lie down. Animals had access to a standard pelleted ration (UAF-RR, Alaska Garden and Pet Supply, Anchorage, AK 99510), which was 18-20 % crude protein, 28 % neutral detergent fiber, 4-5 % acid detergent fiber, and >80 % *in vitro* dry matter digestibility and water. Blood samples were collected starting 17:30 (Zero h) and continued for 18 h. Feed was removed at the start of blood collection. After each blood sample, catheters were flushed with heparinized saline. Animal behaviors i.e. standing and lying were recorded at time of sampling. Blood samples were

collected into plain vacutainers at hourly intervals. Serum was separated and frozen (-20 °C) until assayed.

Laboratory Analysis

Insulin assay: Serum insulin was measured by a solid phase ^{125} I RIA kit (Coat-A-Count Insulin ®, DPC, USA). The radioimmunoassay kit consists of 12 x 75 mm polypropylene assay tubes coated with antibodies to Insulin. ^{125} I- labeled human insulin (concentrate), and 7 concentrations of lyophilized human insulin (0, 5, 15, 50, 100, 200, 400 : IU/ml). The assays were performed as described in the package insert without modification. Insulin standards and the lyophilized ^{125} I tracer were reconstituted with distilled water. Standard solutions, unknown samples and ^{125} I tracer were added to duplicate antibody coated assay tubes. Tubes were mixed and incubated at 20 °C to 24 °C (room temperature) for 18-24 h. Tubes were decanted and radioactivity remaining bound to the tubes was quantified using a gamma counter. Concentrations of insulin in unknown samples were calculated from logit-log representation of the standard curves.

Insulin assay validation: To validate the insulin assay for our species, we determined species specificity, lack of interference, intra-assay and inter-assay precision, and assay sensitivity (Reimers et al., 1981). Species specificity was evaluated with inhibition curves. Curves generated by serial dilutions of sera containing various endogenous concentrations of insulin were examined for parallelism with curves generated by the standard solutions. We tested for heterogeneity of slopes using PROC GLM (SAS

Windows Version 8). Lack of interference from cross-reacting compounds was determined by adding known amounts of insulin to serum samples. Because purified reindeer insulin was not available for this study, purified human insulin in processed human serum (Coat-A-Count Insulin ®, DPC, USA) was added at the following quantities of 25, 50 and 100 μIU/ml to reindeer sera and recovery determined. Intra-assay precision was determined by calculating the coefficient of variation (CV) for 4 replicates of reindeer serum. Inter-assay precision was determined by calculating the CV of 2 pools of reindeer serum in 3 separate, consecutive assays over a period of 3 wk. Serum glucose and lactate concentrations were measured with a glucose-lactate analyzer (2300 Stat Glucose/L-Lactate analyzer YSI®).

Analysis protocols

Insulin, Glucose and Lactate: Individual serum insulin, glucose and lactate profiles over the 18 h period (17:30-11:30) were analyzed for periodicity. The 4:30 sample for W and L could not be included in the analysis due to damage. We excluded the first 2 h serum samples for L from periodic analysis, since the animal appeared to be stressed during the first hour of blood sampling. A sine curve was fitted to residuals obtained from regression of serum insulin, glucose and lactate versus time to test for type of cyclicity. For insulin a sine curve [$Y = a \bullet \sin \bullet (2 \Pi x/b + c]$ best fitted the data. A modified sine curve [$Y = a \bullet \sin (\Pi \bullet (x-x_0/b)]$ was fitted to the residuals for glucose and lactate (Sigma Plot Version 5). We tested for synchronicity between 18 h profiles using correlation analysis. Linear

regressions fitted to individual serum glucose and lactate profiles were compared for homogeneity with respect to slopes and elevation by covariance analysis (Snedecor, 1956) (SAS System Version 8). We ran correlation analysis between the residuals of each variable (SAS System Version 8). Significance level was set at $P < 0.05$.

Results

Animal Behavior

Animals adapted well to the individual stalls and remained calm throughout the 24-h period with the exception of L, who initially was restless and did not settle down for the first hour of bleeding. During the first 18 h animals spent the majority of time standing (M 82 %; L 76 %; W 53 %) with the remainder of the time spent in a laying position.

Insulin assay validation

Slopes did not differ significantly between curves generated by standard solutions and reindeer serum ($F = 0.70$; $P = 0.53$) (Fig.16a), suggesting that serial dilutions of reindeer sera inhibited binding of [^{125}I] iododinsulin to the antibody in a manner parallel to inhibition by purified human insulin in the standard solutions. We concluded that the assay was specific for our species. When various quantities of human insulin were added to samples of serum, essentially a 100 % was recovered (after subtraction of the endogenous concentration) (Fig.16b). The lack of interference from cross-reacting antigens indicates that the assay was accurate for estimation of serum insulin in these

reindeer. The interassay coefficient for 2 reindeer control pools run in 3 consecutive assays was 12 %. Intra-assay coefficient ranged from 2.8-4.0 %. Sensitivity of the assay system was determined to be 4.8 μIU/ml.

Variation in serum insulin and metabolites

Insulin: Mean insulin concentration over 18 h was 39 ± 3 μIU/ml with a baseline range of 27-85 μIU/ml (Table 5). The 18-h serum insulin profile (17:30-11:30) of two animals (W, M) was characterized by a bi-phasic cyclicity, namely a nocturnal rise in serum insulin levels, an early morning trough followed by an early midday peak (Fig.17). Within the larger peaks five short-term oscillations occurring every 2-3 h, were consistently observed for W, but only two were observed for M. Analysis for individual profiles for W and M indicated a period length of 9 h and that a sine curve provided a statistically significant fit (W: $r^2 = 0.49$; F = 7.06; P = 0.0069; M: $r^2 = 0.54$; F = 9.53; P = 0.0019) (Fig.18). For L the profile was characterized by shallow and frequent fluctuations occurring every 2-3 h (Fig.17) and a sine curve analysis indicated a period length of 6 h ($r^2 = 0.49$; F = 5.9; P = 0.017). Correlation analysis indicated that the insulin profiles of the three individuals were not synchronous (r = 0.18; r = 0.02; r = 0.06).

Glucose: Mean serum glucose concentration over the 18 h period was 69 ± 2 mg/dl with a baseline range of 41-64 mg/dl (Table 5). Absolute concentration of glucose during 18 h fast are shown in Appendix 2, Fig. 33. In relation to the fed condition of each animal glucose concentrations increased with time (Fig.19a). Linear regression of glucose

concentration over time for L ($r^2 = 0.80$; P = 0.0001) and M ($r^2 = 0.47$; P = 0.003) were highly significant. The linear model for W. ($r^2 = 0.17$: P = 0.11) was not significant, however glucose concentration also increased in the latter half of the fast following a dramatic decline. A general model for the test for heterogeneity of slopes was significant (df = 5,64; F = 92; P = 0.0001) and there was a significant (P = 0.0002) difference of slopes between animals (Fig.19). Analysis of serum glucose profile indicated that the modified sine curve was statistically significant for two animals (L: $r^2 = 0.41$; P = 0.033; M: $r^2 = 0.51$; P = 0.009) with a period length of 6 h for L and 12 h for M. For W residuals versus time did not vary in a periodic pattern.

Lactate: Mean serum lactate concentration was 26 ± 1 mg/dl with a baseline range of 30 - 44 mg/dl (Table 5). Absolute concentration of lactate during 18h fast are shown in Appendix 2, Fig. 33. In relation to the fed condition of each animal lactate declined with time for L ($r^2 = 0.82$; P = 0.0001), M ($r^2 = 0.45$; P = 0.0045) and W ($r^2 = 0.85$; P = 0.0001) (Fig.19b). A general model for the test for heterogeneity of slopes was significant between animals (df = 5,64; F = 83; P = 0.0001) and there was a significant difference of slopes (P=0.0029). Analysis of serum lactate profiles indicated that a modified sine curve was statistically significant for L with a period length of 15 h ($r^2 = 0.76$; P = 0.0001), for M the period length was 12 h ($r^2 = 0.67$; P = 0.0008) and for W the period length was 10 h ($r^2 = 0.76$; P = 0.0001). Correlation between residuals for glucose with lactate, lactate with insulin, insulin with glucose for individual animals was not significant for L (glucose x lactate r = -0.46; P = 0.075; lactate x insulin r = 0.22; P = 0.43; insulin x glucose r = -0.10; P = 0.72). For M the lactate x glucose correlation was significant (r =

0.93; P= 0.0001), however lactate x insulin (r = -0.28; P = 0.29) and insulin x glucose r = -0.28; P = 0.29) were not significantly correlated. For W the lactate x glucose (r = -0.52; P = 0.039) and lactate x insulin (r = 0.65; P = 0.0006) were significantly correlated, but insulin was not correlated with glucose (r = -0.38; P = 0.145).

Discussion

Mean serum insulin concentration (39 ± 3 μIU/ml; 1.7 ± 0.1 ng/ml) observed in our study was comparable to values observed in September (1.9 ± 0.4 ng/ml) for Norwegian reindeer (Larsen et al., 1985). Serum insulin profiles for all 3 reindeer contained 2-3 h oscillations. In two reindeer the oscillations were superimposed on two distinct serum insulin peaks (arrows in Fig.17), one during nighttime and one during early daytime. In humans and rats, meal initiation has been linked to periodic pulsatile fluctuations in serum insulin and subsequent associated declines in serum glucose concentration (Bray, 2000). Similar data are lacking for ruminant species. In reindeer the short-term fluctuations in serum insulin occurring every 2-3 h have a similar periodicity as the mean intermeal interval length (150 ± 12 min) of pen fed reindeer during winter (Chapter 2).

Although these results support the hypothesis for an endogenous insulin rhythm that could initiate feeding events, the results could also be a function of nutrient supply to the small intestines brought about by rumination. If this occurred the insulin residuals should be correlated with those for glucose or lactate. Analysis of serum glucose and lactate profiles indicated statistically significant periodicity of both glucose and lactate

concentration, but with variable period length between animals. Fluctuations of serum glucose concentration were significantly negatively correlated to serum lactate but were not correlated with insulin. Thus the insulin oscillation is likely not driven by rumination.

Mean glucose concentration from our study (69 ± 2 mg/dl; 3.8 ± 0.1 mmol/L) was higher than autumn and winter levels (2.2-2.5 mmol/L) reported by Nieminen and Timisjaervi (1983) for grazing Finnish reindeer, but was slightly lower than levels previously found in pen-fed male reindeer (4.2 - 5.4 mmol/L, 75-98 mg/dl, McEwan et al., 1976) during early winter. Serum glucose concentrations in reindeer are highly variable and are affected by nutritional plane i.e. pen-fed vs. grazing (McEwan et al., 1976), handling stress (Rehbinder, 1990) and restraint method (Arnemo and Ranheim, 1999). The increase in glucose concentrations over the 18 h period for two animals and an increase in the third after an acute early decline (Fig.19) were unexpected. We do not attribute the increases in glucose concentration to stress, because serum lactate, another indicator of stress, at 26 ± 1 mg /dl (2.8 ± 0.1 mmol/L) was at the lower range of those previously reported for reindeer during winter (2.83-5.16 mmol/L, Nieminen and Timisjaervi, 1983). Also, stress usually results in an elevation of blood lactate rather than the decline noted here.

The unexpected increase in serum glucose levels during short-term feed deprivation suggests that gluconeogenic precursors were being made available for *de novo* glucose synthesis. Sources of glucose carbon include absorbed ruminal propionate, glycerol from fat mobilization, amino acid absorption, protein degradation and interconversion of serum lactate to glucose via the Cori Cycle. The Cori cycle is an

important glucose sparing mechanism during short-term fasting in humans, ruminants, ponies (Anwer et al., 1976; Baird et al., 1980; Baird et al., 1983; Katz and Tayek, 1999) and the rate of recycling of plasma glucose via the Cori Cycle in fed reindeer is very high (Luick et al., 1973; McEwan et al., 1976). Glucose metabolism and the source of serum glucose in reindeer are similar to other ruminants i.e. strictly from de novo synthesis of absorbed nutrients and re-cycling of glucose metabolites (McEwan et al., 1976). An alternative explanation is that tissue utilization of glucose declined throughout the fast, but glucose clearance from blood is driven by concentration and we do not favor this explanation.

In conclusion these results show a periodicity of insulin secretion in the absence of feeding. The periodicity of serum insulin was similar to the feeding behavior in reindeer (Chapter 2) and supports the hypothesis that insulin is one component of an underlying endogenous feeding rhythm. Corroborative data for a melatonin entrained clock mechanism underlying pancreatic secretion exists for laboratory rodents (Peschke et al., 1997; Peschke and Peschke, 1998; Peschke et al., 2000). Since feeding frequency differs during day and night in reindeer (Chapter 2) the light-dark cycle could provide a *zeitgeber* to entrain the hypothesized insulin cycle. A test of the hypothesis that insulin affects the feeding cycle and is under photoperiodic control and thereby drives appetite, would be to administer insulin and to determine its effect on meal patterns, daily food intake and body condition.

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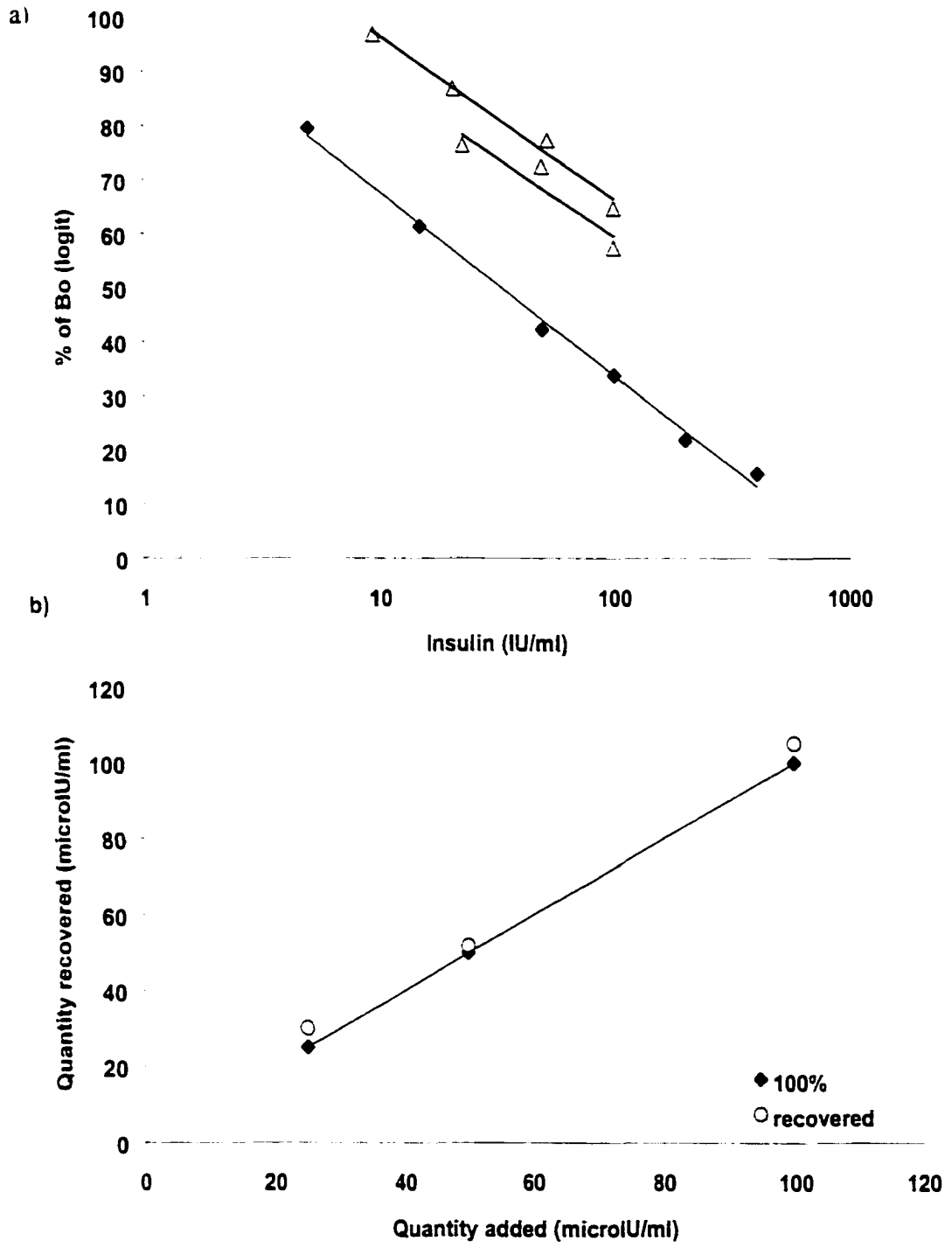

Fig. 16. Inhibition curves for insulin standard solutions (square) and serial dilutions of reindeer sera (triangle) (a). Recovery of human insulin from reindeer sera (b).

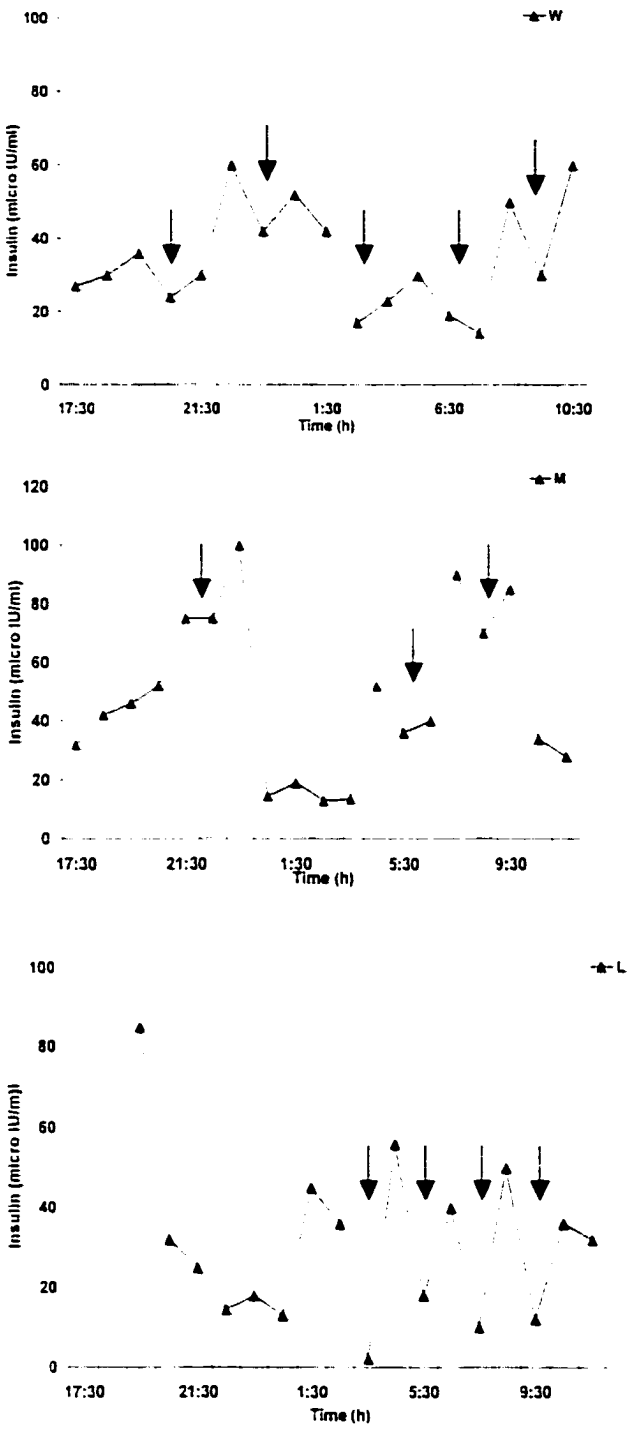

Fig.17 Insulin secretion pattern of three reindeer (W, M, L) during an 18 h fast. Food was withheld from 17:30 (zero hour for sampling). Arrows denote short-term oscillations (1-3 h).

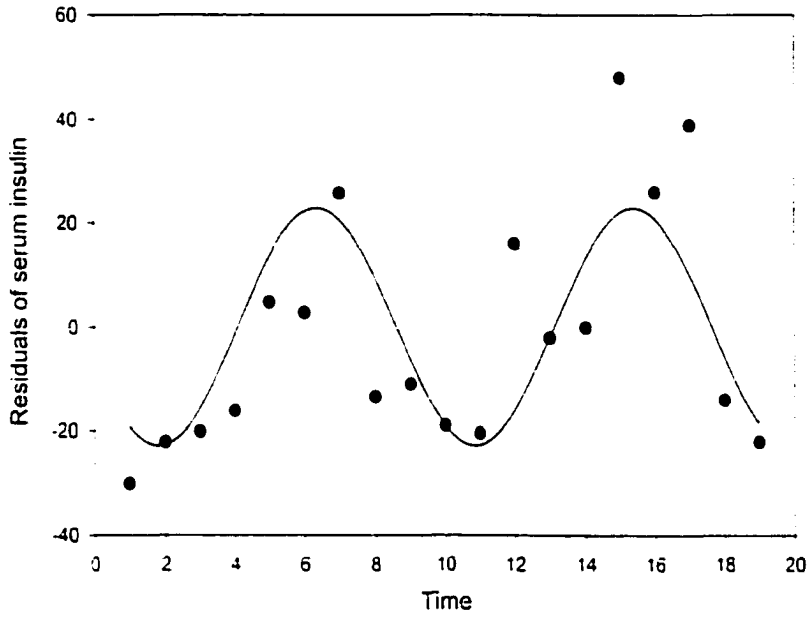

Fig. 18. Example of sine curve fitted to deviations from mean insulin concentration (residuals) during an 18h fast for reindeer M. Curve was given as Y= a● sin ● (2 Π x/b − c. where Y = deviation from mean and X = time since food was withheld (time of fast). Periodicity of peak was 9 h.

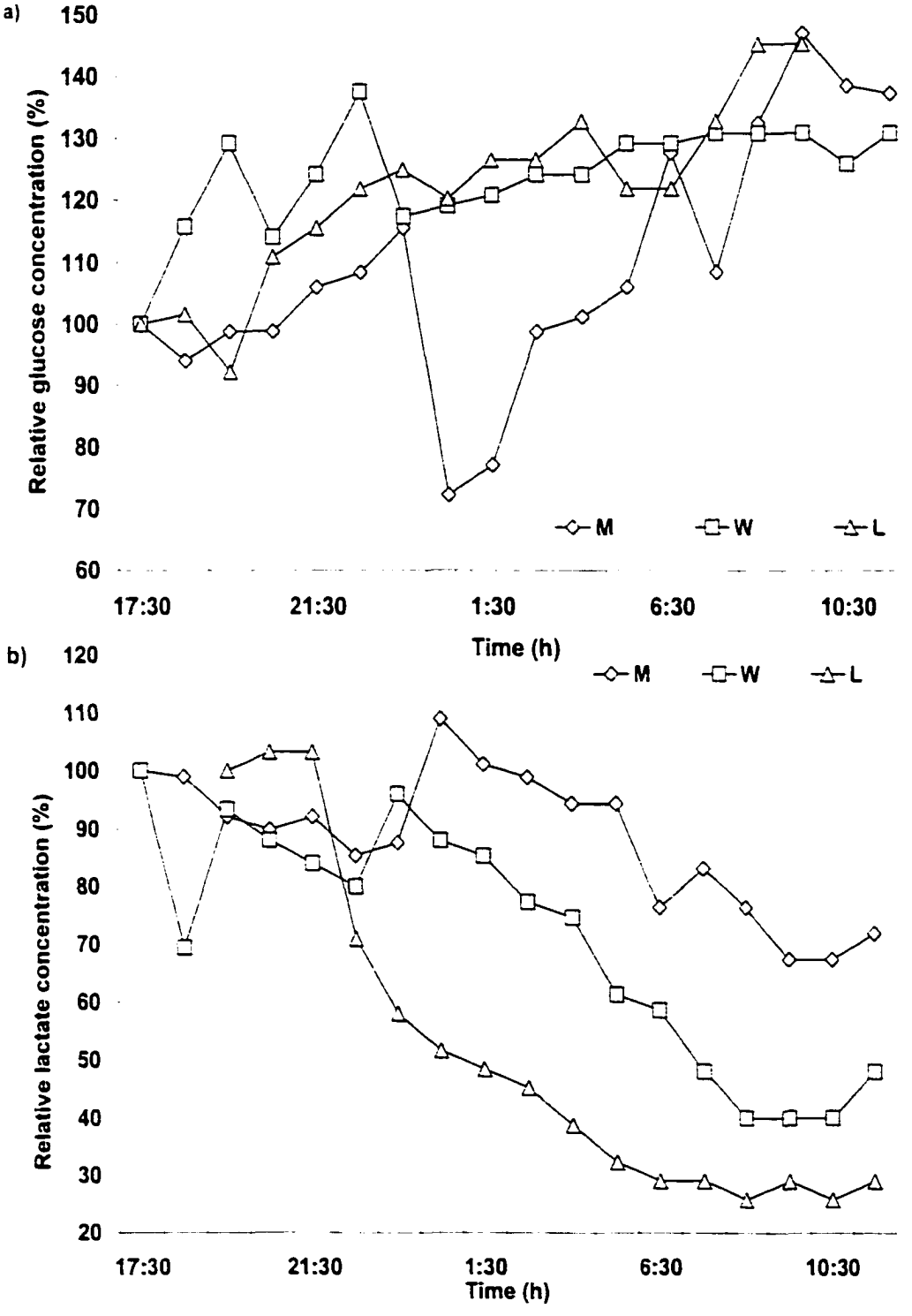

Fig. 19. Relative concentrations of serum glucose (a) and lactate (b) in three reindeer (M, W, L) during an 18 h fast. Food was withheld from 17:30 (zero hour for sampling).

Table 5. Mean and baseline serum insulin, glucose and lactate concentration in three reindeer (W, M, L) initiating an 18 h fast during winter at LARS.

Variables	mean	W	M	L
Insulin (μIU/ml)	39.0 ± 3.0	27.0	32.0	85.0
Glucose (mg/dl)	69.0 ± 2.0	59.4	41.4	64.8
Lactate (mg/dl)	26.0 ± 1.0	37.8	44.1	30.6

Chapter 4

Effects of chronic insulin treatment on body mass, body composition, daily food intake and meal patterns in reindeer[1]

Abstract

This experiment was conducted to determine aspects of the hormonal control over diurnal and nocturnal differences in feeding behavior of reindeer (*Rangifer tarandus t).* Exogenous long acting insulin (1 IU/kg, s.c.) or placebo (Lactate Ringer sol. 0.005 ml/kg s.c.) was administered over a 21 d period daily at 9:00-10:00 to reindeer fed a concentrate diet under natural photoperiod conditions at 64° 49' N, 147° 43' W during early winter. We could not statistically detect an effect of exogenous insulin on serum insulin levels. Exogenous insulin prevented an up-regulation of food intake during a warming trend, and tended to counter a linear decline in body mass and backfat depth (measured by ultra-sound) typified by control animals. Exogenous insulin resulted in a loss of daytime and nighttime differences in meal size and intermeal interval length and a decrease in mean meal size. Thus, based on its effect on intake during the warming trend, insulin exerts a role over appetite, and given the changes in daytime and nighttime feeding behavior, it affects meal size in short-term appetite regulation.

Introduction

We report (Chapter 2) that nighttime meal patterns are different to the regular daytime pattern in reindeer fed a concentrate diet during early winter. Small frequent

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meals during the day were correlated with an incurred energy deficit since time of last meal supporting evidence for a metabolic control over meal size. Following the larger nighttime meals we noted long resting periods, but meal size was not predicted from the daytime meal size energy deficit relationship. Based on these findings we proposed that daily meal pattern regulation in reindeer during winter involves metabolic regulation during daytime while some aspects of the nighttime feeding appears related to a deficit in rumen fill.

Evidence of a metabolic control over meal size led Mayer (1953) to postulate that short-term articulation of energy intake with energy needs is under glucostatic control, and is therefore influenced by insulin. In Mayer's model, hunger drive is considered innate and is down-regulated in the appetite center by a physiological "brake" which constitutes satiety. The "brake" is released due to an energy deficit (low blood glucose) and thereby permits feeding. The glucostatic theory as proposed in 1953 is simplistic and control by both alimentary stretch receptors and hormones are inferred rather than being incorporated mechanistically (Forbes 1996; Bray 2000ab; Szekely 2000). The list of hormones that have been shown to influence voluntary food intake and individual meal size and frequency is extensive and includes insulin, glucagon, leptin, growth hormone, thyroid hormone, glucocorticoids, corticotropin releasing hormone, gonadal steroids and melatonin (Bray 1985; Bray and York 1998; Bray 2000a; Cavagnini et al. 2000; Mystkowski and Schwartz 2000). Of these, insulin (Larsen et al. 1985) has been shown to exhibit pronounced seasonal fluctuations in north temperate ruminants, specifically reindeer. In reindeer serum insulin levels peak during summer during a time of

hyperphagia and maximum body mass gain, and are lowest during winter hypophagia and weight stasis or loss (Larsen et al 1985). Insulin is known to increase food intake in ruminants (Houpt 1974; Deetz et al. 1980; Deetz and Wangsness 1980) by reducing hepatic glucose output and by increasing glucose utilization rate by skeletal muscles (Brockman 1986). Insulin-induced hypoglycemia, studied in other species, results in hyperphagia that does not seem to be mediated by an increase in secretion of hypothalamic neuropeptide Y (NPY) (Corrin et al. 1991; Dryden et al. 1998). Thus insulin is a likely candidate for short-term regulation through effects on meal size.

In non-ruminants insulin has been shown to act as a short-term satiety factor as well as an appetite stimulator. Meal termination appears to be related to a post-prandial rise in insulin triggered by increasing blood glucose levels (VanderWeele 1994; Surina-Baumgartner et al. 1995); and meal initiation follows a typical post-meal decline in glucose concentration (Campfield 1997). The latter is preceded by a rise in insulin suggesting that insulin secretion could be initiating the sequence. When the transient glucose decrease is prevented, the following meal is delayed (Louis Sylvestre & LeMagnen 1980; Campfield et al. 1985; Melanson et al. 1999) lending support for a meal-initiating role for insulin. There is evidence that pre-meal insulin peaks could be of endogenous origin, possibly originating from a pancreatic pacemaker (Peschke et al. 1997; Peschke and Peschke 1998; Peschke et al. 2000). Thus for non-ruminant species insulin is associated with the initiation of feeding events and with the size of meals.

Because dietary carbohydrates are largely fermented to short-chained volatile fatty acids (VFA) and little glucose is absorbed, it might be expected that a glucostatic-

insulin control system is unlikely to occur in ruminants. Yet a rise in circulating insulin is observed following feeding in ruminants and is linked to the post-prandial rise in ruminal VFA (Matsunaga et al. 1999). Thus insulin could play a role in structuring meal size in ruminants. Ultradian oscillations in serum insulin secretion occur in the fasting reindeer (Chapter 3). The period of the oscillations is similar in duration to mean intermeal intervals estimated for pen-fed reindeer during winter (Chapter 2). Thus although not causal, the results suggest a possible role for insulin in meal initiation.

These findings argue for a function of insulin in the short-term regulation of food intake in ruminants. A test of the hypothesis that endogenous insulin is involved in appetite regulation would be to study effects of insulin on daily food intake and meal pattern in a species that exhibits a strong endogenous down regulation of daily food intake and a relative tight linkage between energy intake and energy requirements under *ad libitum* feeding conditions. This occurs in reindeer during the winter short photoperiod (Suttie et al. 1991).

We designed a study in which long acting recombinant human insulin was administered daily for a 21 d period to reindeer during January. At the onset, we predicted that insulin treatment would improve glucose, and probably VFA utilization, and thus reduce inter-meal interval and increase meal frequency. In light of our findings of few and large nighttime meals which drive the nocturnal meal patterns (Chapter 2), we hypothesized for nighttime meals that elevated insulin levels will reinforce the "brake " on feeding (Mayer 1953). The predicted outcome was more regular nighttime feeding and a decrease in meal size. The objective of this study was to examine effects of long-

term insulin treatment on (i) voluntary food intake, (ii) an index of rumen fill and daily meal patterns to clarify insulin's role in short-term regulation of daily food intake, (iii) intake-related effects on body mass and body composition indices, and (iii) serum insulin, glucose and lactate concentrations to give insight into physiological mechanisms.

The study was approved by the Institutional Animal Use and Care Committee at the University of Alaska Fairbanks.

Material and methods

Animals and measurements

Eight adult non-pregnant reindeer were used for feeding behavior studies during winter 1996-1997 (16 Dec. - 13 Feb). Animals were held at the Large Animal Research Station (LARS), Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks (64° 49' N, 147° 43' W) and had free access to a standard pelleted ration (UAF-RR, Alaska Garden and Pet Supply, Anchorage, AK 99510), which was 18-20 % crude protein, 28 % neutral detergent fiber, 4-5 % acid detergent fiber, and > 80 % *in vitro* dry matter digestibility. Snow was freely available. Maximum and minimum daily temperature was recorded at the weather station of the Agricultural Forestry Experiment Station, University of Alaska Fairbanks, which is approximately 2 km from the study site. Animals used in this study were exposed to regular handling prior to the experiment to minimize stress.

Experimental Design

Reindeer were randomly allocated (14 Jan.) to insulin or placebo treatment. Group I received 1.0 IU/kg • d ultralente insulin (Ultralente Humilin®) for 21 days. Group II received an equivalent volume of placebo (Lactate Ringers solution). Insulin or placebo injections were administered subcutaneous in the pre-scapular area daily at 09:00-10:00. In previous ruminant studies insulin injections exceeding 1 IU/kg resulted in severe hypoglycemia with subsequent convulsions (Houpt 1974). To reduce the risk of inducing severe hypoglycemia we chose (i) a long-acting human insulin suspension with zinc, which provides a slower onset and less intense duration of activity (PDR 2001); and (ii) a dose of 1 IU/kg which is at the lower range of previously reported dosages used in feeding behavior studies in ruminants. In addition blood glucose concentrations were monitored with dipsticks prior to daily injections to check for hypoglycemia.

Laboratory Analysis

Blood samples were collected daily from each animal prior to injections from the jugular vein and kept chilled (4-8 °C) until centrifuged. Serum was separated and kept frozen (-20 °C) until analyzed.

Insulin: Insulin was measured by a solid phase ^{125} I RIA kit (Coat-A-Count Insulin ®, DPC, USA) validated for our species (Chapter 3). The assays were performed as described in the package insert without modification (Chapter 3). Concentrations of insulin in unknown's samples were calculated from logit-log representation of the standard curves. Intra-assay precision was determined by calculating the coefficient of

variation (CV) for 4 replicates of reindeer serum. Interassay precision was determined by calculating the CV of 2 pools of reindeer serum in 3 separate, consecutive assays over a period of 3 weeks. The interassay coefficient for 2 reindeer control pools run in 3 consecutive assays was 12 %. Intra-assay coefficient ranged from 2.8-4.0%. Sensitivity of the assay system was determined to be 4.8 IU/ml. Initial values (pre-treatment period) for insulin were compared with Student t-test (SAS System Version 8). Data were averaged for individual animals over 5-day periods. Data were analyzed as two-factor designs with a repeated measure on time (SAS System Version 8). Significant treatment-time interactions were followed by a one-way ANOVA for each treatment and subsequent contrast post-hoc comparison (SAS system Version 8). Fitting linear and quadratic regression curves to the means of control and treatment group data summarized temporal fluctuations in serum insulin during treatment period. Regression equations were tested for homogeneity of slopes and elevations using covariance analysis (SAS System Version 8.)

Glucose and Lactate: Serum glucose and lactate concentrations were measured with a glucose-lactate analyzer (2300 Stat Glucose/L-Lactate analyzer YSI®). Data were averaged for individual animals over 5-day periods. Data were analyzed as two-factor designs with a repeated measure on time (SAS System Version 8). Significant treatment-time interactions were followed by a one-way ANOVA for each treatment and subsequent contrast post-hoc comparison (SAS system Version 8). Fitting linear and quadratic regression curves to the means of control and treatment group data summarized temporal fluctuations in serum glucose and lactate during treatment period. Regression

equations were tested for homogeneity of slopes and elevations using covariance analysis (SAS System Version 8).

Body mass and backfat depth

Body mass (BM): All animals were weighed daily (nearest 0.5 kg) using an electronic balance.

Backfat depth (BFD): BFD, an index of body fat (Chapter 1) was measured on alternate days (nearest 0.1 cm) with a real-time portable scanner (Technicare Model 210DX) along a longitudinal line between the tuber coxae and the tuber ischii (Chapter 1). Data were averaged for individual animals over a 5 d period. Initial values (pre-treatment period) for BM and BFD were compared with Student t-test (SAS System Version 8). To test for differences in BM and BFD between group's data were analyzed as two-factor designs with a repeated measure on time (SAS System Version 8). Significant treatment-time interactions were followed by a one- way ANOVA for each group and subsequent contrast post-hoc comparison. Fitting linear and quadratic regression curves to the means of control and treatment group data summarized the temporal fluctuations in BM and BFD during treatment period. Regression equations were tested for homogeneity of slopes and elevation using covariance analysis (SAS System Version 8).

Feeding behavior

Daily dry matter intake (DDMI): All animals were held as a mixed group and fed communally. Each day at 09:00-11:00 two individuals were placed in adjacent individual

pens with modified feed bins to monitor 24 h feeding behavior. Daily food intake was determined as food offered minus refusals (measured to nearest 1 g) and daily food intake was corrected for dry matter content of fresh food by drying a sample to 100 °C in a convection oven for 24 h (Suttie et al. 1991). Because of the known effect of ambient temperature on DDMI (for review Kennedy et al. 1986), we adjusted the observed DDMI to that at the mean ambient temperature (-18°C, 255 °K) (temperature-adjusted DDMI) using the equation [1]:

[1] $$Y = 3450 - 7.99 \bullet X$$

Where Y = DDMI (g) and X = mean daily temperature (°K) (Blanchard 1983).

Data were averaged for individual animals over a 5 d period. Initial values (pre-treatment period) for DDMI and temperature-adjusted DDMI were compared with Student t-test (SAS System Version 8). To test for differences between groups data were analyzed as two-factor designs with a repeated measure on time (SAS System Version 8). Significant treatment-time interactions were followed by a one-way ANOVA for each treatment and subsequent contrast post-hoc comparison. Temporal fluctuations in DDMI and temperature adjusted DDMI during treatment period were summarized by fitting linear and quadratic regression curves to the means of control and treatment group data. Regression equations were tested for homogeneity of slopes and elevations using covariance analysis (SAS System Version 8).

Meal size and inter-meal interval: Two feeding pens were equipped with an automated feed monitor consisting of a feed bin attached to a weighing plate of an electronic balance

(Model 610T, Arlyn Scales, Lynbrook, NY) as reported earlier (Chapter 2). In brief, the voltage output of each balance was continuously monitored every 5 min throughout the 24-h period, and data were stored automatically onto a computer. A program was written to determine time of onset and completion of each eating event, food eaten (g) and the inter-meal interval. Data were accepted only if the apparatus recorded the start and endpoint of a feeding event (Chapter 2).

Daily feeding activity cycles and photoperiod

Hourly distribution of number meals per hour (meals/h) and mean meal size per hour (g/h) observed were calculated from original data sets from 24-h feeding activity over 21 d for the control and treatment group. We analyzed the 24-h data for rhythms for the control and treatment group as in Chapter 2. To test for type of cyclicity of daily feeding activity a sine curve was fitted to (i) the residuals of daily distribution of number of meals/h data set, and (ii) the residuals of daily distribution of mean meal size/h (Sigma Plot SPSS Version 5) for the control and treatment groups. We assumed that if insulin injections influence daily distribution of feeding behavior then the patterns should be asynchronous between treatment and control group. We used correlation analysis to test for asynchronicity (SAS System Version 8).

Timing of feeding activity: We made the assumption, since reindeer time the beginning of feeding activity to sunrise (Chapter 2) that little feeding activity should be observed prior to sunrise and an increase in feeding activity should occur at or near sunrise. If daily insulin injections affect timing of the beginning and ending of feeding activity to sunrise

then feeding activity should be different between control and treatment groups. To test for differences in timing of feeding activity we compared number of meals for the 3 h period pre-sunrise as well as for the 3 h post sunrise for each group using Wilcoxon test (SAS System Version 8.).

Meal Pattern Analysis

Data sets from 24-h feeding activity over 21 d for control and treatment group were calculated as: number of meals/d, meal size distribution (g) 50-100, 101-175, 176-250, 251-325, 326-400,401-475,476-550, inter-meal interval distribution in 30 min increments (0-570 min), and the inter-meal interval distribution in 7 meal-size intervals. To account for diurnal and nocturnal feeding activity we separated meal events by daytime and nighttime. A minimum cut-off of 50 g was used as the criterion for a meal (Baile 1975). Amounts of food consumed of less than 50 g were considered "nibbling" events. To test the general hypothesis that insulin affects daily meal patterns by decreasing meal size and intermeal interval the data were examined in relation to daylight and darkness (Sibbald 1994). Daily feeding frequency and mean meal size/d were compared between groups using a Student' t test and a Wilcoxon test (SAS System Version 8). Daytime and nighttime differences in total number of meals/h of daytime and nighttime and mean meal size of daytime and nighttime between groups were compared using two-way analysis of variance with an unbalanced design (SAS System Version 8). Significant treatment-time interactions were followed by a one-way ANOVA for each treatment and subsequent contrast post-hoc comparison. Daily meal size distribution and

daytime nighttime differences were compared between groups using a Fisher's exact test. respectively Chi-square test. Where small-expected cell values occurred (< 5), meal size classes were collapsed (SAS System Version 8). Daily distribution of intermeal intervals and daytime nighttime differences in mean intermeal interval were compared between groups using a Chi-square test. respectively a two-way ANOVA with an unbalanced design. Significant treatment-time interactions were followed by a one-way ANOVA for each treatment and subsequent contrast post-hoc comparison (SAS System Version 8).

We tested the effect of insulin on the relation between meal size and the energy deficit incurred since the last meal (Chapter 2. Baile. 1975) and Campling's model in which the rate of processing of food controls meal size and time between feeding events (Campling 1970). Mean meal size for each size class was related to the mean intermeal interval (pre and post) associated with the size classes (Baile 1975). All regressions are reported with the significance of the model. Meal size was regressed on both a theoretical energy deficit (ED) calculated from time since the previous meal, and a theoretical rumen-fill deficit (RD) calculated from time since the previous meal (Chapter 2). Regression equations were tested for homogeneity of slopes and elevations using covariance analysis (SAS System Version 8). A final test of the ED and RD models developed for control animals was to apply them to those given insulin treatment. We used the observed daytime and nighttime intermeal interval to predict daytime and nighttime meal size for the ED and RD models within each treatment group and compared these results with observed values.

Results

Photoperiod and Temperature

Mean total possible sunlight was 5 h 37 min ± 15 min. with a minimum of 4 h 24 min h and a maximum of 6 h 53 min (3 Feb.). Total possible sunlight increased throughout the study period (Fig. 20). Mean temperature was 0.9 ± 3 °C with a minimum of -30.5 °C (26 Jan) and a maximum of 18 °C (14 Jan.). The temperature profile over the study period was characterized initially with temperatures above freezing followed by a cold period with temperatures reaching - 30 °C and a warming trend to above freezing by the end of the study (Fig. 20).

Laboratory Analysis

The two groups had comparable initial (pre-treatment) values for serum insulin. glucose and lactate concentrations (Table 6). however trends over the ensuing 21 d were variable and required time dependent tests to assess effects of insulin injection. Absolute concentration of hormone and metabolites are shown in appendix 3. Fig. 34.

Insulin: There was no significant *time x treatment* interaction for insulin between groups (P = 0.717) (Table 7). Fitting quadratic regression models to mean insulin over treatment period was not significant for control (P = 0.25) and insulin groups (P = 0.46) (Table 8) (Fig. 21).

Glucose and Lactate: There was no significant *time x treatment* interaction for glucose between groups (P = 0.324) (Table 7). Fitting linear regression models to mean glucose over treatment period was not significant for control (P = 0.089) and insulin group (P =

0.76) (Table 8)(Fig. 22a). There was no significant *time x treatment* interaction for lactate between groups (P = 0.070)(Table 7). However there was a trend for serum lactate levels to decrease after 10 days of treatment (Fig. 22b) Fitting linear regression models to mean serum lactate over treatment period was not significant for control (P = 0.264) and just failed to reach significance for the insulin group (P = 0.069) (Table 8).

Body mass and backfat depth

The two groups had comparable initial values for BM. and BFD (Table 6). Absolute measurements of body mass and backfat depth are shown in appendix 3. Fig. 35.

Body mass: There was no significant *time x treatment* interaction for BM between groups over the time period (Table 7). Fitting linear regression models to mean BM over treatment period for control and insulin group indicated that there was a significant decline in BM over the treatment period for both control (P = 0.04) and insulin groups (P = 0.018) (Fig. 23) (Table 8). Overall model test of heterogeneity of slopes was not significant (F = 0.35; P = 0.758).

BFD: The *time x treatment* interaction term for BFD was significant (P = 0.046) (Table7). One-way ANOVA for insulin group indicated that there was a significant time effect (df = 4,12; F = 3.92; P = 0.029). Pair-wise contrast comparison indicated that BFD after 5 d treatment was significantly smaller than pre-treatment value (P = 0.034) (Fig. 24c). After ten days of treatment the difference was no longer significant (P = 0.077). One-way ANOVA for control group was not significant (df = 4,12; F = 2.15; P = 0.172).

Fitting (linear regression models to mean BFD over treatment period was not significant for control (P = 0.31) and insulin group (P = 0.154) (Table 8).

Feeding behavior

Animals adapted well to the individual pens and ate regularly within the individual pen. Nibbling was not observed. A total of 23 twenty-four hour feeding activity data sets (control n = 10; treatment animals n = 13) were recorded during the 21 d study period. Total numbers of meals analyzed were 175 with 75 for control animals and 100 for treatment animals.

Daily dry matter intake (DDMI): The two groups had comparable initial values of DDMI and there was no significant difference between groups (Table 6). Absolute measurement of DDMI and temperature-adjusted DDMI are shown in appendix 3. Fig. 36.

DDMI: The *time x treatment* interaction for DDMI marginally failed significance (P = 0.058) (Table 7). There was a trend for observed DDMI to decrease after 5 d of insulin treatment with an apparent recovery after 10 d of treatment (Fig. 25a). Fitting linear regression models to mean DDMI over treatment period was not significant for control (P = 0.13) and insulin group (P = 0.177) (Table 8).

Temperature-adjusted DDMI: The *time x treatment* interaction for temperature-adjusted DDMI marginally failed significance (P = 0.055) (Table 7). Fitting linear regression models to mean temperature-adjusted DDMI over treatment period was not significant

for control (P = 0.217), but was significant for insulin group (P = 0.019) (Fig. 25b) (Table 8). Temperature-adjusted DDMI increased over time for insulin group.

Daily feeding activity cycles

Daily meal activity pattern for the study period was characterized by two active peaks; one during daytime (10:00-17:00) and a broad peak during nighttime (18:00 - 06:00) (Fig. 26a). The end of nighttime feeding activity and beginning of daytime feeding activity was separated by about a 4-h period of no (control) or low (insulin) feeding activity. The distinction between end of daytime feeding activity and the beginning of nighttime feeding activity was less distinct with a 1-h intermittent period of low feeding activity. Twenty-four hour profiles were highly synchronous (r = 0.77; P = 0.001), but peaks for the insulin group reached higher amplitude, in particular during the early nighttime period. Analysis for daily distribution of meals/h indicated that a sine curve of the type Y = a•sin (pi•(x-x0)/b) was an appropriate fit for both groups (control: $r^2 = 0.34$; df = 2,20; F = 5.17; P = 0.017; insulin: $r^2 = 0.28$; df = 2,20; F = 3.84; P = 0.039). Diurnal and nocturnal feeding activity extended each for approximately 10-11 h periods with troughs between diurnal and nocturnal feeding activity at between 06:00-7:00 and 18:00-19:00. Daily distribution of mean meal size per hour of day for the control group was characterized by a decline in average meal size from midnight towards the early morning hours and a subsequent gradual rise in meal size over the course of the day with the largest meals occurring during nighttime (Fig. 26b). The latter rise appeared blunted in the insulin group. Correlation analysis indicated that 24-h profiles between groups were

marginally not synchronous (r = 0.38; P = 0.067). Analysis of daily distribution of meal size at time of day indicated that a sine curve of the type Y = a•sin (pi•(x-x0)/b) was an appropriate fit for the control (r^2 = 0.60; df = 2,20; F = 15.93; P = 0.0001) and insulin (r^2 = 0.26; df = 2,20; F = 3.7; P = 0.043) groups. Period lengths were 14 h and 4 h for control and insulin groups respectively.

Timing of feeding behavior sunrise: Mean (± SEM) number of meals in the 3 h period prior to sunrise (1 ± 0.7) was not significantly different from the 3 h period post sunrise (5 ± 2.3) (Wilcoxon; one-tailed t-approximation P = 0.121) for the control group. Likewise for the treatment group mean number of meals in the 3 h period prior to sunrise (1 ± 0) was not significantly different from the 3 h period post sunrise (6 ± 3.3) (Wilcoxon; one-tailed t-approximation P = 0.127).

Meal Pattern Analysis

Feeding frequency: Mean (± SEM) number of meals per day for control animals are 7.5 ± 0.5 (range 4 -10) and were not significantly different from treatment animals 7.7 ± 0.5 (range 5 -11) (Student t-test: df = 21; P = 0.798). Mean (± SEM) meal size for the treatment group (132 ± 8 g) was smaller than controls (153 ± 10 g) (Wilcoxon test: P = 0.042). Comparison of meals/h during daytime and nighttime indicated (df = 3,44; F = 3.24; P = 0.0311) that there was no significant *treatment x time of day* interaction term (P = 0.72). There was a significant time of day effect (P = 0.0083) with more meals/h during daytime (control: 0.4 ± 0.1; insulin 0.5 ± 0.1) than nighttime (control: 0.20 ± 0.04;

insulin: 0.27 ± 0.03) (Table 9). This result suggests distinctive diurnal and nocturnal feeding activity in both groups. Comparison between daytime and nighttime meal sizes between groups indicated a significant *treatment x time of day* interaction term (F = 3.77; P = 0.054). Comparing the treatment effect on daytime and nighttime meals size (F = 2.85; P = 0.03) indicated that for the control group mean nighttime meal size (174 ± 14 g) was significantly larger than mean daytime meal size (127 ± 14 g), but there was no significant difference between mean daytime (134 ± 13 g) and nighttime meal size (130 ± 11 g) for the insulin group. Mean daytime meal size did not differ significantly between insulin and control group, but mean nighttime meal size for the insulin group was significantly smaller than for the control group (Table 10).

Meal size distribution: Control and treatment groups were characterized by small meals (88 % versus 95 % of meals < 250 g) with respectively 12 % versus 5 % of meals > 251 g) for control and treatment group (Table 10). Meals size distribution did not differ significantly between groups (Fisher's exact test; two-sided P = 0.101). No treatment effect was found on daytime and nighttime composition of meals for insulin (42 % vs. 58 %) and control groups (44 % vs. 56 %) (Chisquare; Chi = 0.07; P = 0.791). There was no effect of treatment on daytime and nighttime composition of meals (< 250 g) between insulin group (42 % vs. 58 %) and control group (45 % vs. 55 %). For daytime and nighttime distribution of meals (> 251 g) 75 % of cells had expected counts less than 5 (Table 10). For the control group the largest meals (> 326 g) occurred during nighttime and for the insulin group the largest meal (> 401g) occurred during daytime. Percent of

total meals (Y) and meal size (X, g) were related for control Y = 46.13 - 8.42 •X (r^2 = 0.89; df = 1,4; F = 32.5; P = 0.005;) and treatment group Y = 51.47 - 9.94 •X (r^2 = 0.77; df = 1,4; F = 13.5; P = 0.021;). Regression equations did not differ significantly in slopes (P = 0.612) and elevation (P = 0.974) (Fig. 27a).

Inter-meal interval (mean, pre- and post-meal interval): Mean (± SEM) inter-meal interval for control animals was 150 ± 12 min and not significantly different from 156 ± 13 min for treatment animals (Student t-test; df = 150; T = -0.35; P = 0.73). Inter-meal intervals ranged between 30-480 min for control animals and 30-515 min for treatment animals with the majority of the inter-meal intervals being < 300 min for control animals (90 %) and treatment animals (97 %) (Fig. 27b). There was no significant difference between groups for inter-meal interval distribution (> 30 < 270 min) (Chisquare; df = 3; F = 6.4; P = 0.095). Pre-meal interval within 7 meal size intervals ranged for control animals between 123 - 308 min and between 141 - 265 min for treatment animals (Table 10). Post-meal interval within 7 meal size intervals ranged for control animals between 111 - 213 min and 105 - 225 min for treatment animals (Table 10). Comparison between daytime intermeal interval and nighttime intermeal interval between groups indicated a highly significant *treatment x time* of day interaction term (F = 8.7; P = 0.004). Comparing the treatment effect on daytime and nighttime intermeal interval (F = 4.2; P = 0.007) indicated that there was no significant difference between mean daytime (159 ± 21 min) and nighttime intermeal interval (153 ± 16 min) for the insulin group, but for the control group mean nighttime intermeal interval (197 ± 16 min) was significantly larger

than mean daytime intermeal interval (103 ± 15 min). Mean daytime intermeal interval for the insulin group was significantly larger than the control group. Mean nighttime interval for the control group was significantly larger than for the insulin group (Table 9).

Pre-meal interval: Meal size (Y, g) correlated with pre-meal interval (X, min) for the control group ($Y = 1.49 \bullet X - 50.5$, $r^2 = 0.80$; df = 1,4; F=12.0; P = 0.041) (Fig. 28a.) and insulin group ($Y = 2.6 \bullet X - 264.9$, $r^2 = 0.91$; df = 1,4; F = 30.2; P = 0.012). Regression equations were not significantly different in slopes (P = 0.15) and elevations (P = 0.984). *Post-meal interval*: Linear regression of post-meal interval (Y, min) on meal size (X, g) was not significant for the control group ($r^2 = 0.01$; df = 1,3; F = 0.03; P = 0.88) and treatment group ($r^2 = 0.01$; df =1,3; F = 0.03; P = 0.87) (Fig. 28b). A quadratic model was significant for the control group ($Y = 34.42 + 2.196 \bullet X + (-0.005) \bullet X^2$, $r^2 = 0.98$; df = 2,2; F = 58.2; P = 0.017), but not for the insulin group ($Y = -0.001 \bullet X2 - 0.508 \bullet X + 114.96$, $r^2 = 0.13$; df = 2,2; F = 0.14; P = 0.87). For the control group a maximum post-meal interval was noted at meal size of 200 g. In the control group post-meal interval declines with either a decrease or increase in meal size about 200 g. In the insulin group a right-shift occurs such that the post-meal interval is maximal at a meal size of 400 g.

Deficits in energy and rumen-fill

Energy deficit: Mean (± SEM) ED (60 ± 5 kJ/kg $^{0.75}$) for the control group was not significantly different from the treatment group (65 ± 6 kJ/kg $^{0.75}$) (Student t-test; df =

152; T= 0.57; P = 0.28) Mean ED (60 ± 5 kJ/kg $^{0.75}$) created by the pre-meal interval for the control group was not significantly different from meal MEI (66 ± 5 kJ/kg $^{0.75}$) (Student t-test; df = 132; T = 0.81; P = 0.21). For the treatment group mean ED (65 ± 6 kJ/kg $^{0.75}$) created by the pre-meal interval was significantly larger than meal MEI (51 ± 3 kJ/kg $^{0.75}$) (Student t-test; df = 129; T = 1.96; P = 0.026). Linear regression of meal MEI (Y) on ED (X) was significant for control (Y = 1.4074 •X - 12.6, r^2 = 0.85; df = 1,3; F = 17.5; P = 0.025) and treatment group (Y = 2.2646 •X - 88.4, r^2 = 0.87; df = 1, 3; F = 20.2; P = 0.021) (Fig.29a). The intercepts were not significant for control (P = 0.69) and treatment (P = 0.12) groups. A quadratic regression for the control group was significant and explained 97 % of the variation (Y = -0.0235 •X^2 + 6.0013 •X - 202.44, r^2 = 0.97; df = 2,2; F = 33.3; P = 0.03). For the insulin group a quadratic regression was marginally not significant (Y = -0.0381 •X^2 + 8.9989 •X - 369.21, r^2 = 0.95; df = 2,2; F = 18.2; P = 0.052). Regression equations did not significantly differ in slopes (P = 0.548), but there was a significant difference in elevation (P = 0.033).

Deficit in rumen-fill: Initial values for max. RF (kg DM) did not differ significantly between control and insulin group (Table6). Mean (± SEM) RD (kg DM) for control group (0.234 ± 0.018) was not significantly different from the treatment group (0.248 ± 0.019 kg DM) (Student t-test; df = 150; T = 0.50; P = 0.62). Mean RD created by the pre-meal interval for the control group was significantly larger than mean meal size (0.157 ± 0.008 kg DM) (Student t-test; df = 128; T = 3.7; P = 0.0002). Mean RD created by the pre-meal interval for the treatment group was significantly larger than mean meal size (0.128 ± 0.006 kg DM) (Student t-test; df = 172; T = 5.8; P < 0.0001). Linear regression

of meal size (Y) on RD (X) was significant for control group ($Y = 1.769 \bullet X - 0.2534$, $r^2 = 0.89$; df = 1,3; F = 25.0; P = 0.02) and insulin group ($Y = 1.1766 \bullet X - 0.1401$, $r^2 = 0.85$; df = 1,3; F =16.9; P = 0.03) with intercepts not significant for control (P = 0.08) and insulin group (P = 0.23) (Fig. 29b). Linear regression equations did not differ significantly in slopes (P = 0.99) and elevation (P = 0.096).

Daytime and nighttime meal size predictions from energy deficit and the deficit in rumen fill (Table 11) indicated that for the insulin treated group ED closely predicted daytime and nighttime meal size in contrast to the control group where nighttime meal size was better predicted by rumen deficit.

Discussion

The ultralente insulin used in our study has a reported extended activity up to 28 h in humans (PDR 2001) and we expected that the sampling protocol (24 h basis) would record any elevations in serum insulin due to insulin injections. However serum insulin concentration did not differ significantly between control and treatment groups. Absence of a significant effect on serum insulin levels suggests that either the insulin dose was cleared from blood in less than 24 h or that exogenous ultralente insulin suppressed endogenous insulin secretion, thus resulting in no net effect on insulin concentrations. Exogenous insulin-induced suppression of endogenous insulin secretion by short-acting and long-acting insulin is documented for laboratory animals and humans in *in vivo* and *in vitro* studies (Beischer et al. 1979; Stagner et al.1986; Koiter et al. 1995; Lindstrom et al. 2000). However, in humans ultralente insulin has been shown to increase meal

induced insulin secretions and favor energy retention as indicated by weight gain (Coutant et al. 2000). More frequent sampling in our study might have detected effects of ultralente insulin injections on meal-elicited insulin secretion. However, frequent sampling would interfere with meal patterns and was thus avoided. Analysis of meal patterns and food intake suggests that exogenous ultralente insulin affected feeding behavior and BFD dynamics.

Serum glucose did not differ between the treatment and control group and remained stable over time. An increase in glycemia over the study period, despite day-to-day variation in DDMI between control and treatment groups, is indicative of a balance between glucose de-novo synthesis and glucose utilization rates being maintained by glucose-carbon recycling (McEwan et al. 1976). The trend for declining lactate concentrations over the study period, particularly for the treatment group, suggests to us the possibility of increased gluconeogenesis from lactate via glucose-lactate cycling (Cori cycle) (Katz and Tayek 1999) brought about by the insulin treatment.

There was no significant difference in body mass between control and treatment groups. Both groups significantly declined in body mass over the treatment period with the mean body mass change ranging between 0.3 - 0.9 kg. The small change in mean body mass over the 3 wk period is typical for reindeer in winter (McEwan and Whitehead 1970; Blanchard 1983). BFD differed significantly between treatment groups. BFD for the control remained constant over the treatment period suggesting maintenance of body fat reserves; however, BFD declined after 5 d of treatment with a subsequent recovery by day 10 of treatment suggested fat deposition in the insulin treated group. Lipogenesis in

reindeer is thought to be absent in winter because lipogenic capacity of adipose tissue is minimal (Larsen et al. 1985ab; Larsen and Nilsen 1985). Our findings suggest that insulin treatment appears to restore lipogenic capacity of adipose tissue. Nutrients and hormones such as insulin have been shown to regulate lipogenic enzyme (i.e. fatty acid synthase) gene expression in adipose and hepatic tissue (Girard et al. 1994). Therefore it is likely that the low lipogenic capacity of adipose tissue during winter in reindeer may be linked to the seasonally low serum insulin levels.

Any effect of insulin on DDMI was expressed as a difference in the control and treatment response to the warming and cooling trends that occurred during the study. The trend for decreasing DDMI during the cold spell (21-30 Jan.) in the insulin treated group was opposite to the control group, suggesting that temperature induced changes in appetite response are somehow modulated by insulin. Chronic cold exposure has been shown to affect glucose-stimulated insulin secretion (decline) and tissue insulin sensitivity (increase) in sheep (Sasaki and Weekes 1986).

In agreement with our findings in Chapter 2 feeding behavior for control animals followed a distinctive diurnal pattern with a period length of feeding activity of 10-11 h, with more meals occurring during daytime than nighttime. Difference in daytime and nighttime meals/h were maintained for insulin treated animals, suggesting that insulin did not have an effect on distribution of meals/h in relation to daytime and nighttime. In contrast insulin treatment eliminated the diurnal-nocturnal difference in meals size by significantly reducing nighttime meal size. Therefore insulin restored responsiveness of the appetite center to a "brake" during nighttime. The findings of shorter intermeal

intervals during nighttime again support the hypothesis that exogenous insulin restores daytime feeding that is balancing metabolizable energy intake with the energy deficit incurred since the previous meal. Although insulin may have increased the sensitivity of meal size response in relation to changes in pre-meal interval, analysis of post-meal interval indicated that mean meal sizes that brought about a maximum post-meal interval differed between groups. This suggests that insulin disrupted the relationship of post-meal interval to meal size that was observed in control animals. In agreement with findings in Chapter 2, in the control group meal MEI was related to ED and meal size was related to RD. However when treated with insulin, we found that MEI was related to ED, but meal size was not related to RF. This supports the hypothesis that exogenous insulin restores a daytime type feeding behavior to the nighttime.

In conclusion our results support previous data on energy intake to energy requirements during winter being tightly regulated in this north temperate ruminant. Given *ad libitum* food a naturally occurring loss in body condition and mass is minimal. Thus changes brought about by insulin treatment on these parameters needed to be very pronounced for us to detect them. Observed insulin effects on DDMI and body fat were subtle in comparison to previous results with rats and humans (Mozes et al. 1978; Larue-Achagiotis et al. 1983; Larue-Achagiotis et al. 1985; Larue-Achagiotis et al. 1988; Woodward and Emery 1989; Roberst et al. 1994; Coutant et al. 2000). However, a trend for DDMI to decrease during a cold spell (21-30 Jan.) exhibited by insulin treated animals was opposite to the control group. Dynamics in backfat indicate that lipogenesis occurred in insulin treated animals and suggest insulin can restore the seasonal depression

of lipogenic capacity in reindeer during winter. Taken in concert we proposed a role for insulin in meal size regulation in reindeer and we show that regular meal eating might be maintained by insulin injections, especially at night. Since the main effects of feeding behavior were restricted to nighttime feeding we speculate on the involvement of melatonin as a hormonal factor responsible for decoupling meal interval - meal size relationships. Corroborative data in human and rats suggest a modulator role for melatonin on insulin secretion and sensitivity, as well as on glucose metabolism (Peschke et al. 1997; Peschke and Peschke 1998; Van Cauter 1998; Peschke et al. 2000). How insulin is involved in satiety responses in relation to other afferent satiety signals requires further investigation. A combination of rhythmic variation in satiety response to meals and decoupling of meal size and frequency at night is suggested as an endocrine model underlying daily appetite regulation in the reindeer.

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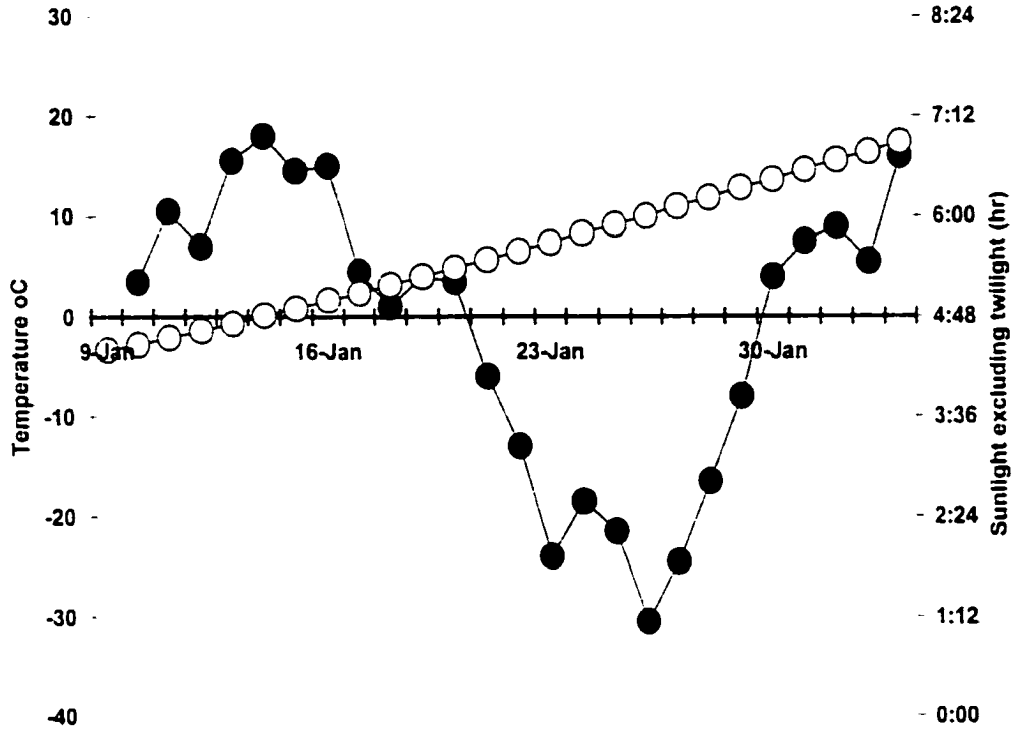


Fig. 20. Temperature profile (•) and total sunlight possible (o) recorded at the Agricultural Experiment Station, University of Alaska Fairbanks 1997.

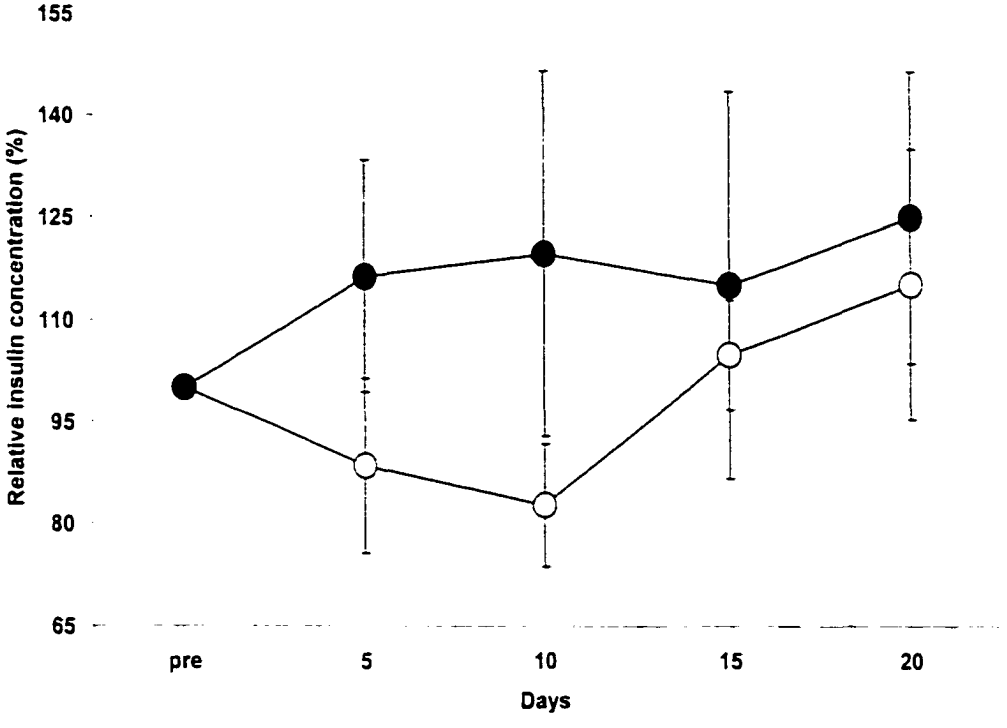


Fig. 21. Serum insulin concentrations for control (n = 4) (o) and insulin treated (n = 4) (•) reindeer over a 21 d treatment period. Data are expressed as % of pre-drug values. Bars represent SEM.

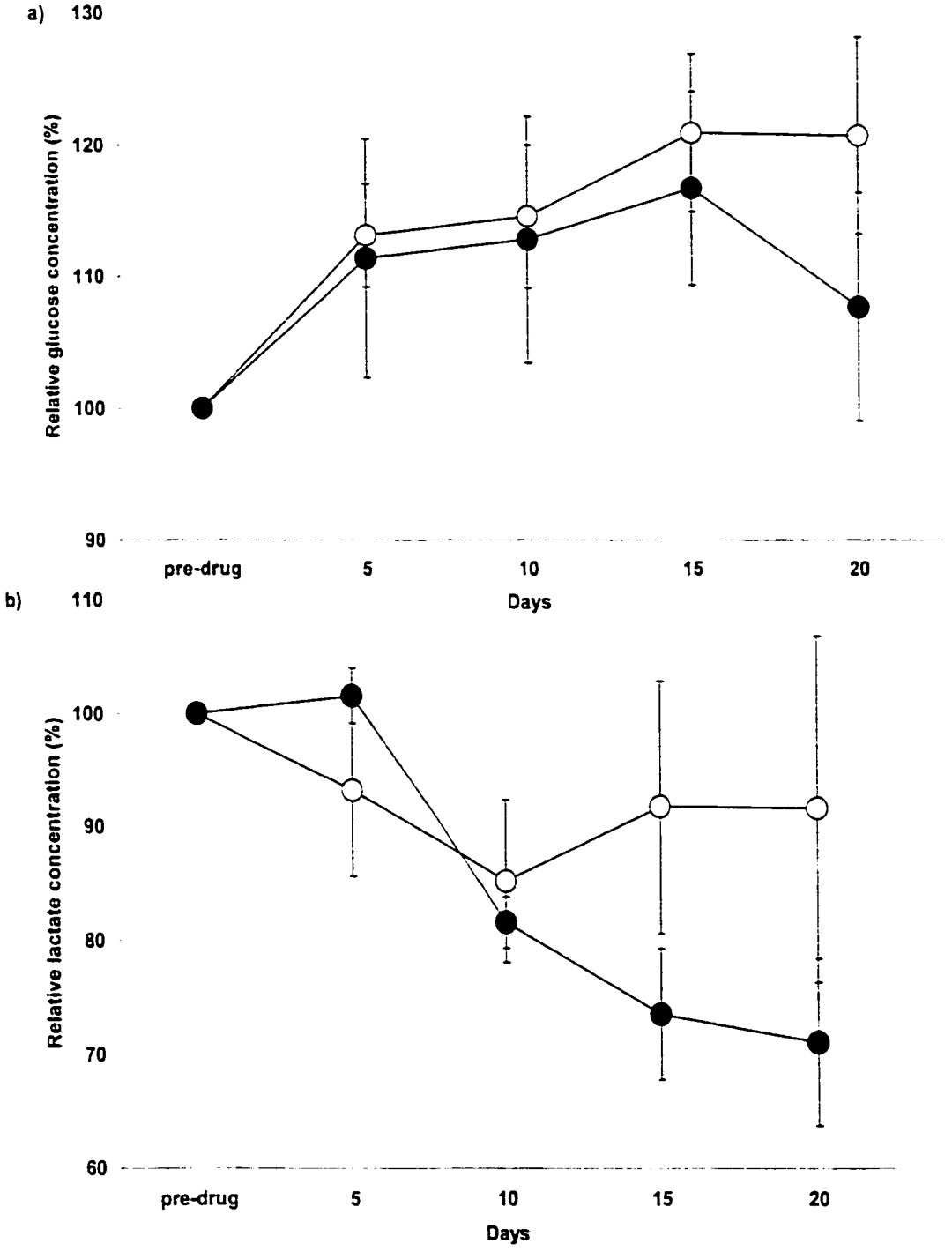


Fig. 22. Serum glucose (a) and lactate (b) concentrations for control (n = 4) (o) and insulin treated (n = 4) (•) reindeer over a 21 d treatment period. Data are expressed as % of pre-drug values. Bars represent SEM.

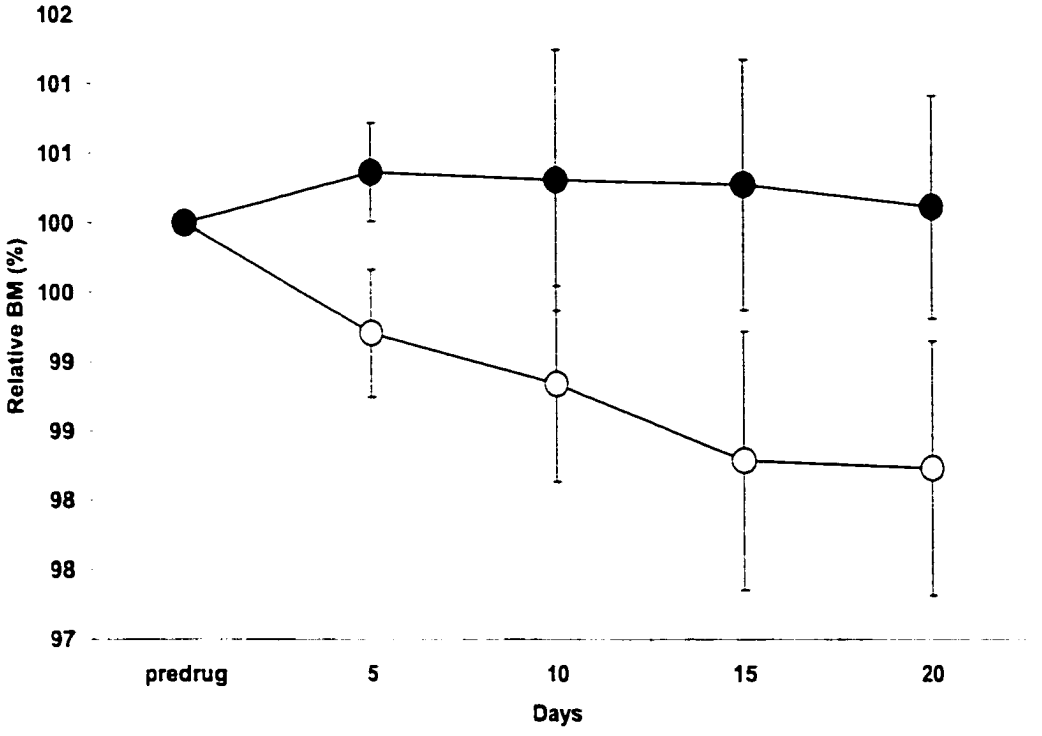


Fig. 23. Body mass (BM) for control (n = 4) (o) and insulin treated (n = 4) (•) reindeer over a 21d treatment period. Data are expressed as % of pre-drug values. Bars represent SEM.

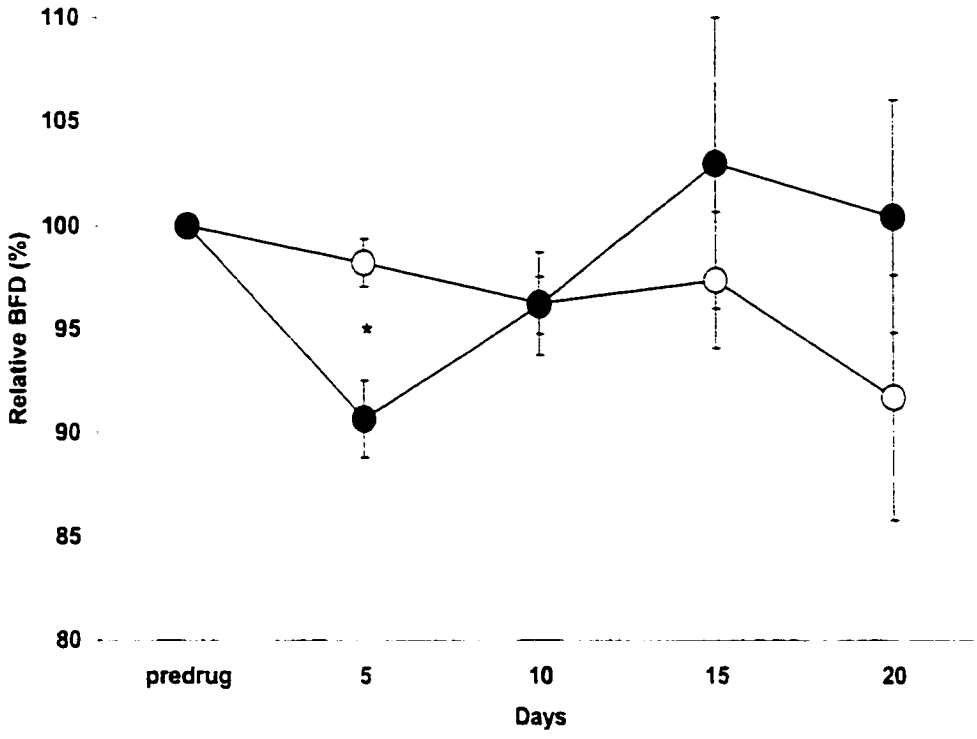


Fig. 24. Backfat depth (BFD) for control (n = 4) (o) and insulin treated (n = 4) (•) reindeer over a 21 d treatment period. Data are expressed as % of pre-drug values. Bar represent SEM. *Significance $P < 0.05$.

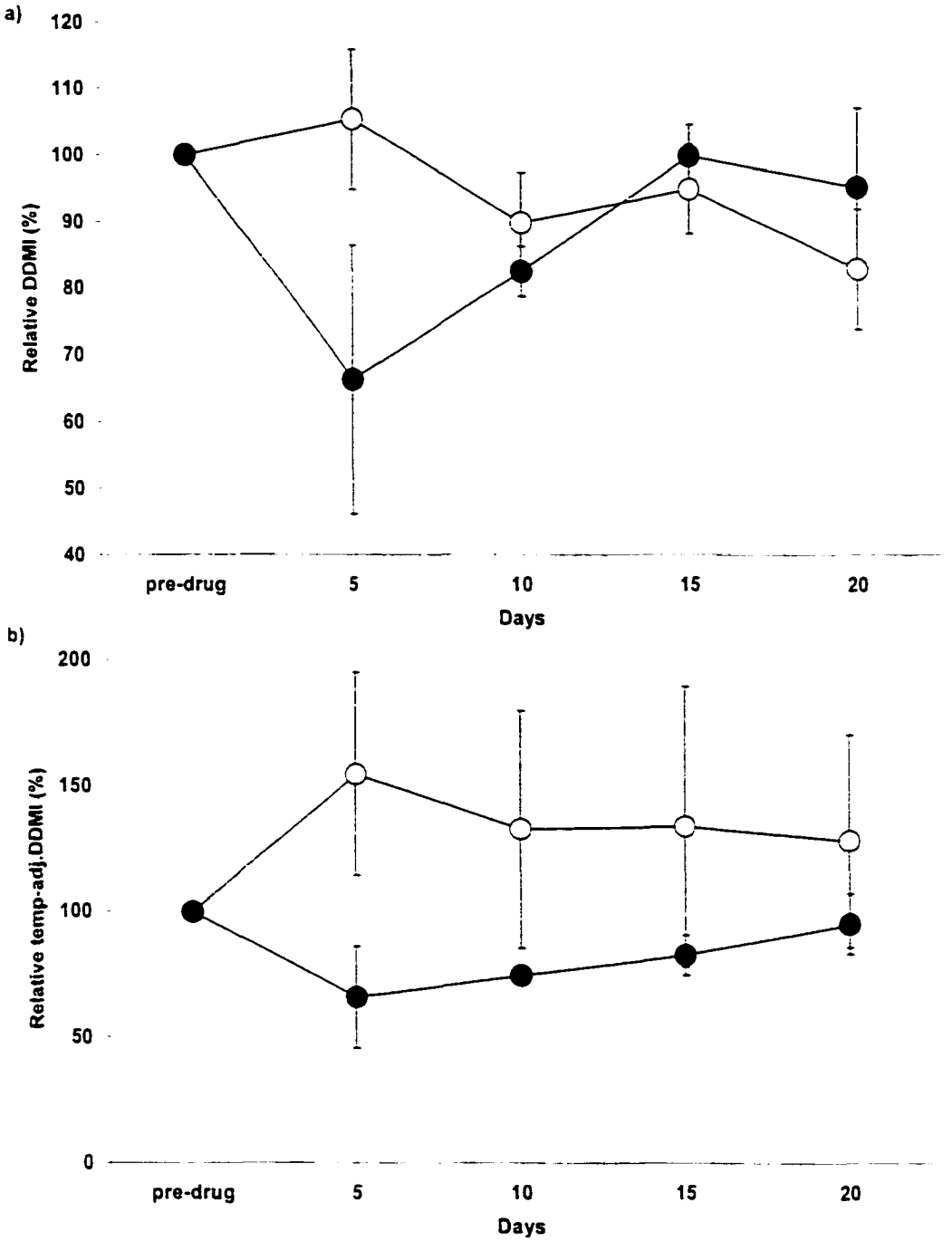


Fig. 25. Daily dry matter intake (DDMI) (a) and temperature adjusted DDMI (b) for control (n = 4) (o) and insulin treated (n = 4) (•) reindeer over a 21 d treatment period. Data are expressed as % of pre-drug values. Bars represent SEM.

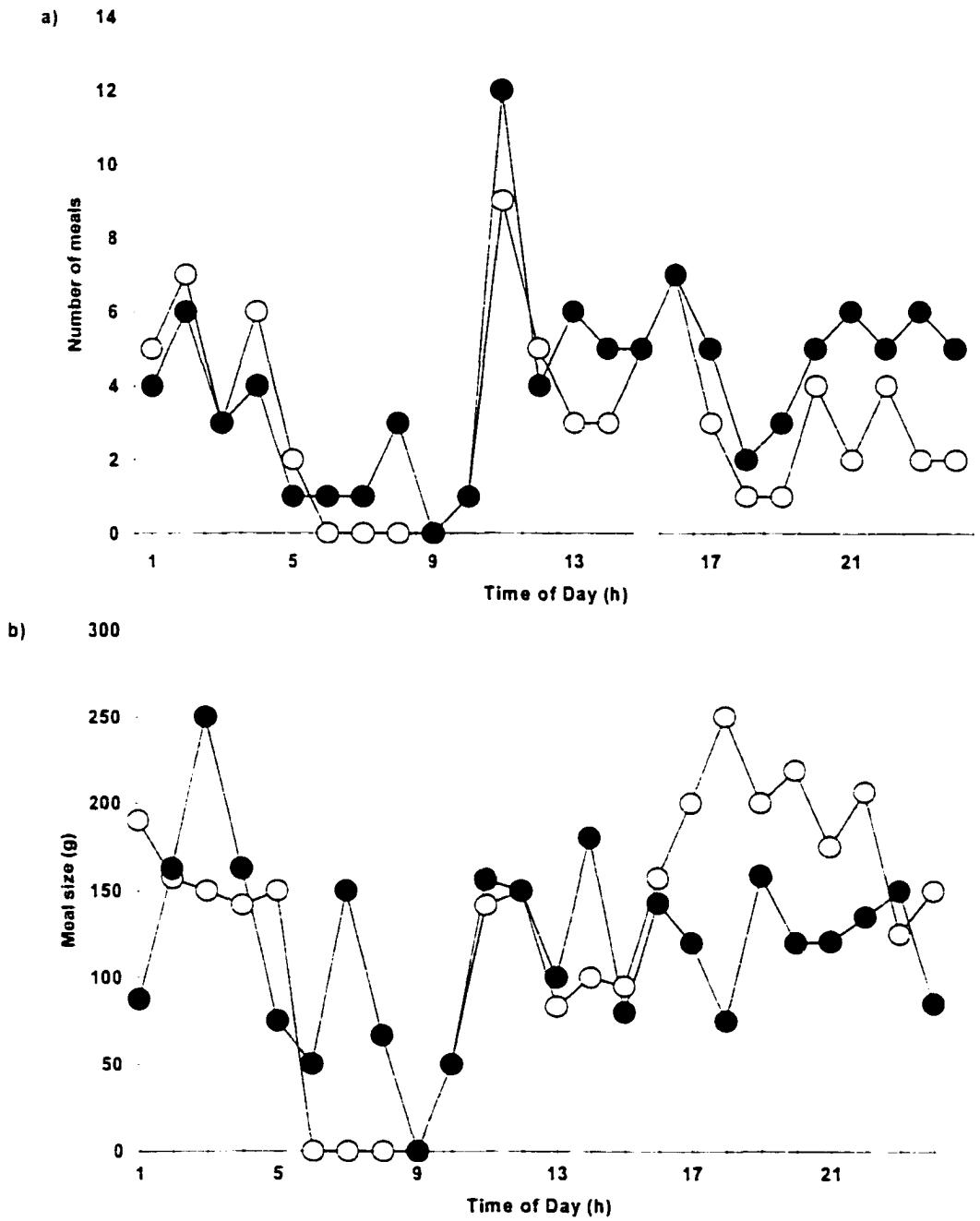
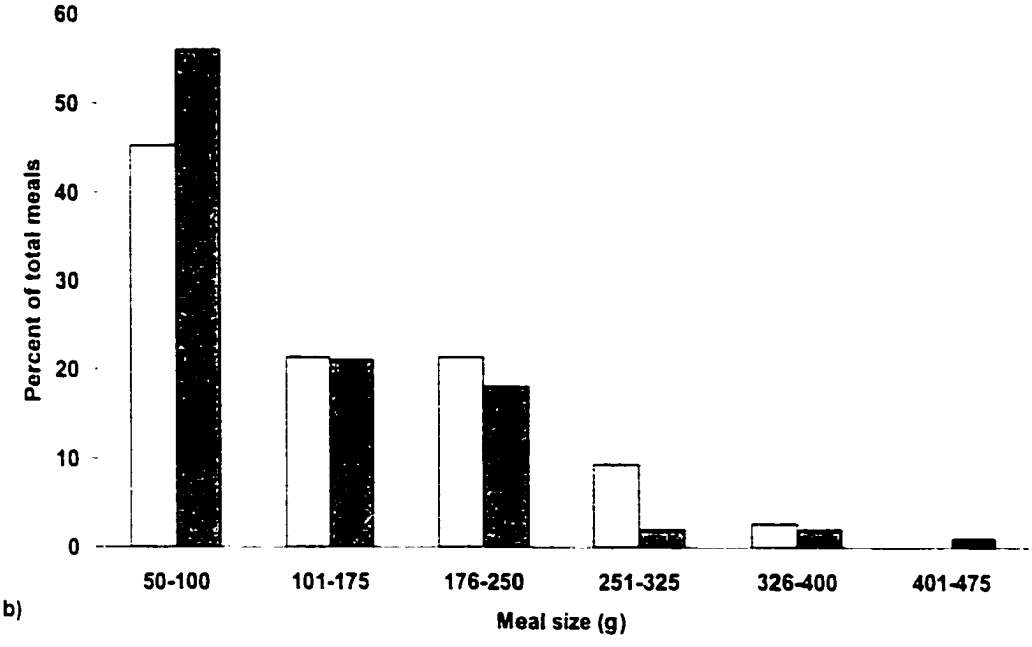


Fig. 26. Meal frequency/h (a) and mean meal size/h (b) for control (n = 4) (o) and insulin treated reindeer (n = 4) (•) distribution over the 24-h of the study period.

a)



b)

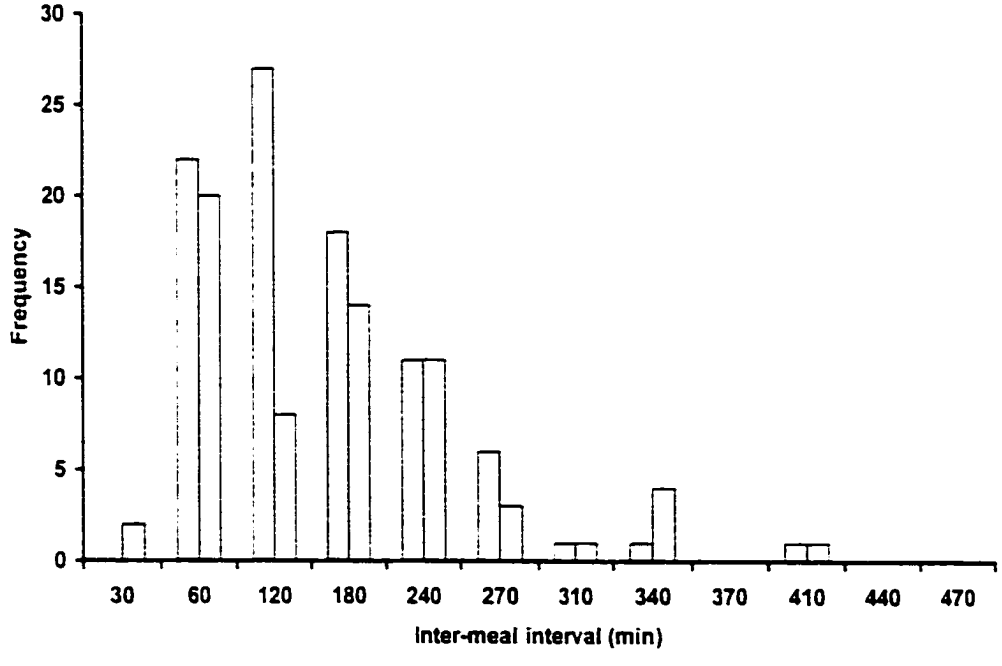


Fig. 27. Meal size distribution (a) and daily intermeal interval distribution (b) for control (n = 4) (open bars) and insulin treated (n = 4) (shaded bars) reindeer.

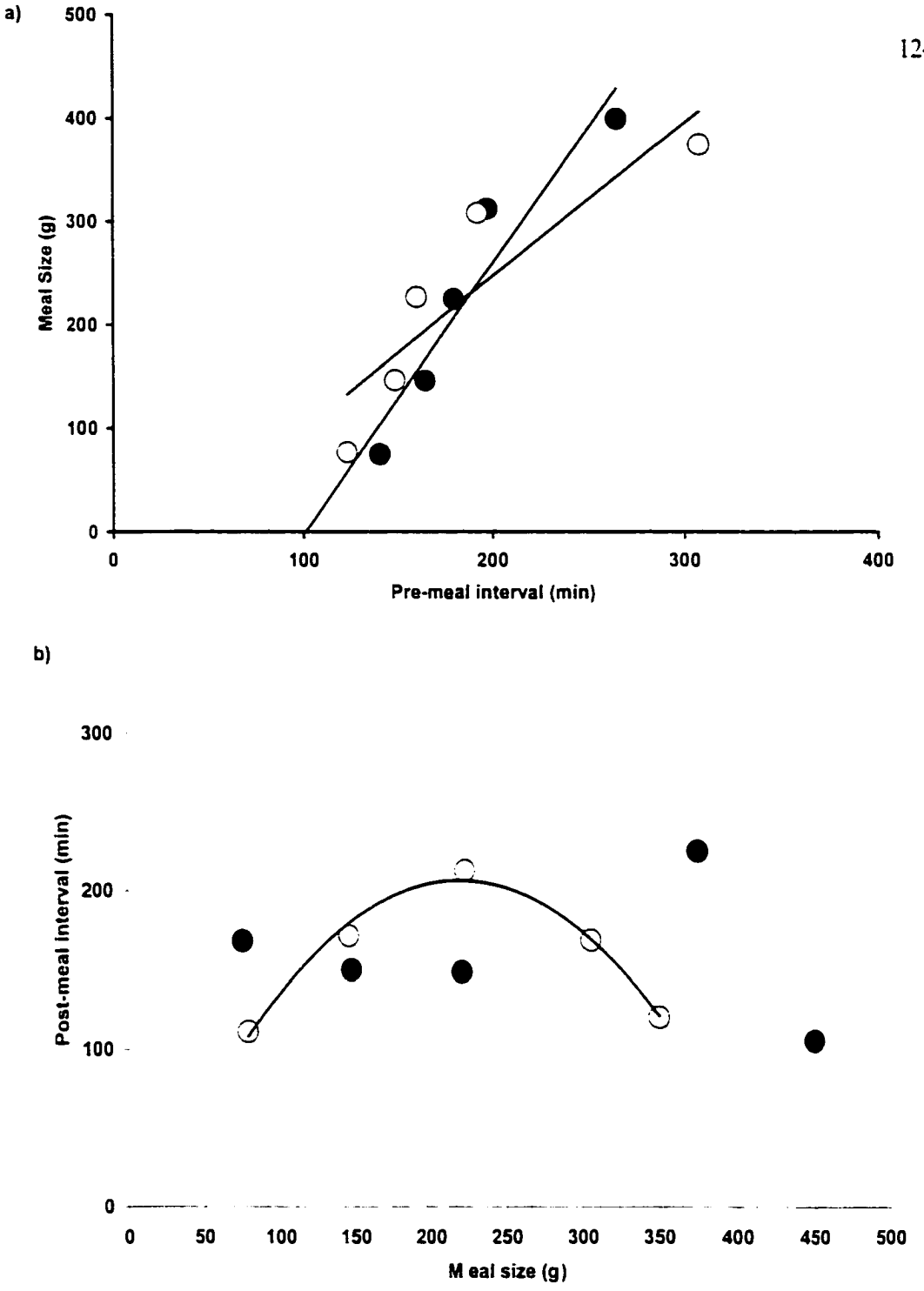


Fig. 28. Relations between meal size and pre-meal interval (a) and between post-meal interval and meal size for control (n = 4) (o) and insulin treated (n = 4) (•) reindeer. See Materials and Methods for definitions.

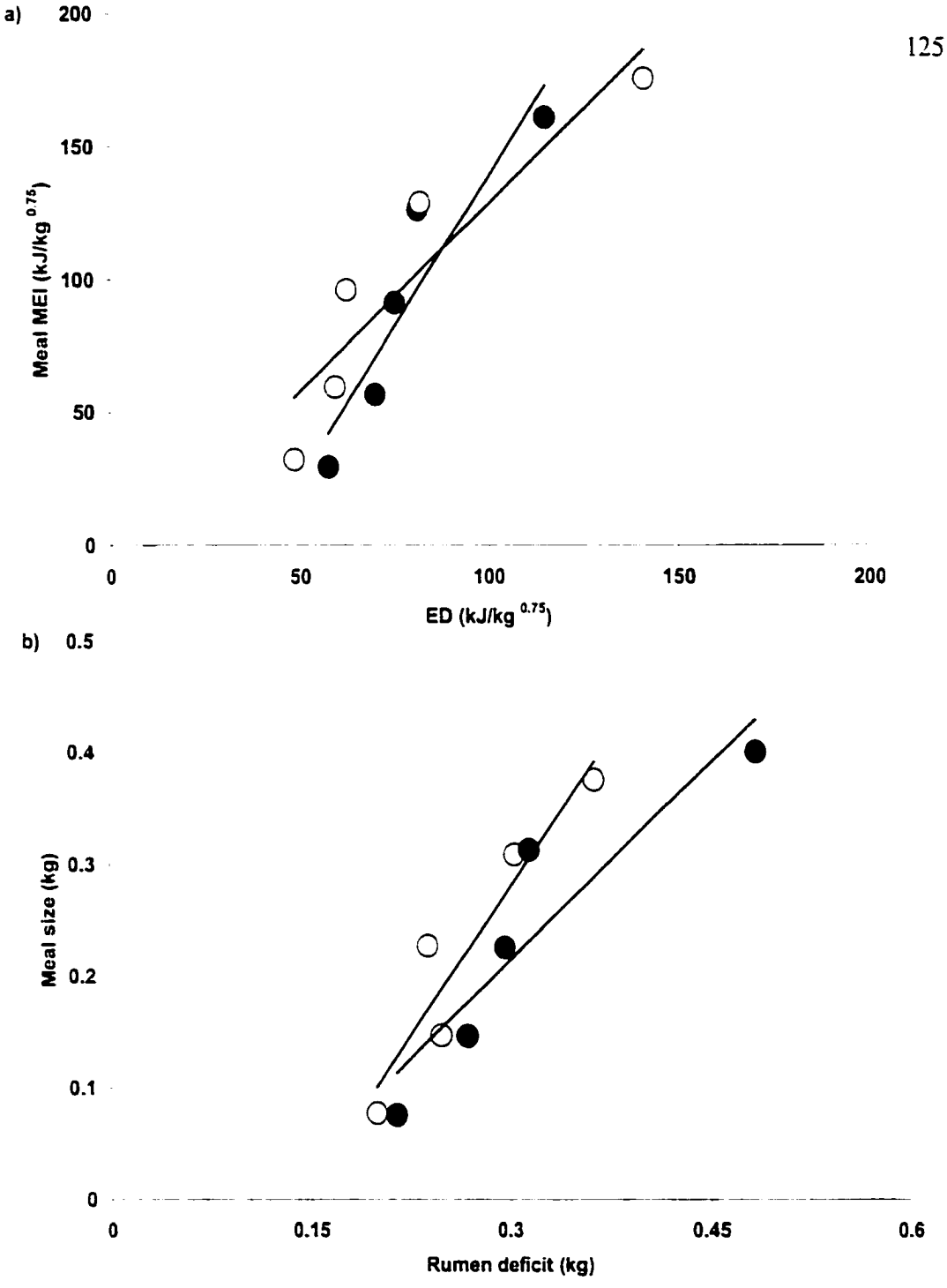


Fig. 29. Relations between meal metabolizable energy intake (MEI) and energy deficit (ED) (a) and between meal size and rumen deficit (RD) (b) for control (n = 4) (o) and insulin treated (n = 4) (•) reindeer. See Materials and Methods for definitions.

Table 6. Comparison of body mass, backfat depth, feeding, hormone and metabolic variables in control and treatment animals during the pre-treatment period (4 -13 Jan.) See Materials and Methods for definitions.

Variables	Insulin (n =4)	Control (n = 4)	P-Value
Body mass (kg)	103 ± 4.6	101 ± 5.2	0.718
Backfat depth (cm)	3.9 ± 0.6	4.4 ± 0.6	0.157
Feeding			
DDMI (kg)	1.21 ± 0.176	1.27 ± 0.109	0.772
temp.adj.DDMI (kg)	1.21 ± 0.176	0.99 ± 0.202	0.459
k-rate	1.20 ± 0.12	1.29 ± 0.07	0.555
max. rumen fill (kg)	2.25 ± 0.094	2.18 ± 0.120	0.339
Hormone & metabolites			
Insulin (μIU/L)	22 ± 4	16 ±2	0.316
Glucose (mg/dl)	81 ± 7	71 ± 4	0.241
Lactate (mg/dl)	35 ± 2	28 ± 3	0.129

Note: Data are expressed as mean ± SEM. P-value determined with Student t-test and Wilcoxon. P-value level of significance < 0.05.

Table 7. RMANOVA tests on the effect of chronic insulin treatment (1 IU/kg body weight) in non-pregnant female reindeer (control n = 4; insulin treated n = 4) during winter on body mass, backfat depth, feeding, hormone and metabolites. For definitions see Materials and Methods.

Time x treatment interaction	$F_{4,24}$	P-value
Body mass (kg)	0.68	0.609
Backfat depth (cm)	2.84	0.046 *
Feeding		
DDMI (kg)	2.65	0.058
Temperature-adjusted DDMI (kg)	2.69	0.055
Hormones and metabolites		
Insulin (μIU/ml)	0.53	0.717
Glucose (mg/dl)	1.23	0.324
Lactate (mg/dl)	2.49	0.070

Note: * P-value significance level < 0.05.

Table 8. Regression equations fitted to means of body mass, backfat depth, feeding, hormones and metabolites. over the treatment period (14 Jan-3 Feb.) for control (n = 4) and insulin treated animals (n = 4). For definitions of variables see Materials and Methods.

		CONTROL				TREATMENT			
Variable	Model	r^2	df	F	P	r^2	df	F	P
Body mass	X	0.92	1,2	23.3	0.040	0.97	1,2	55.8	0.018
Backfat depth	X	0.47	1,2	1.8	0.310	0.71	1,2	5.04	0.154
DDMI	X	0.76	1,2	6.41	0.130	0.67	1,2	4.21	0.177
temp.adj.DDMI	X	0.61	1,2	3.17	0.217	0.96	1,2	49.15	0.019
Insulin	X^2	0.93	2,1	7.19	0.250	0.79	2,1	1.87	0.460
Glucose	X	0.82	1,2	9.8	0.089	0.05	1,2	0.12	0.760
Lactate	X	0.54	1,2	2.37	0.264	0.87	1,2	13.01	0.069

Note: Significance $P < 0.05$.

Table 9. Comparison of daytime and nighttime number of meals, meal size, and inter-meal interval in control (n = 4) and insulin treated animals (n = 4) during the treatment period (14 Jan. - 3 Feb.) Total number of meals analyzed 175 (control n = 75; insulin n = 100). For definitions see Materials and Methods.

	Control		Insulin	
Variables	Daytime	Nighttime	Daytime	Nighttime
Number of meals/h	0.40 ± 0.1	0.20± 0.04*	0.50 ± 0.1	0.27 ± 0.03*
Meal size (g)	127 ± 14	174 ± 14 *#	134 ± 13	130 ± 11
Inter-meal interval (min)	103 ± 15	197 ± 16*#	159 ± 21#	153 ± 16

Note: * indicates significant difference within group in which P is < 0.05 using a 1-way ANOVA with contrast post-ANOVA test.

# indicates significant difference between control and treatment group in which P is < 0.05 using a 1-way ANOVA with contrast post-ANOVA test.

Table 10. Meal size distribution for control and treatment group. A total of 175 meals were analyzed (control = 75; insulin = 100).

	Meal Size Classes					
Control	50-100	101-175	176-250	215-325	326-400	401-475 476-550
% of total meals	45	21	21	9	3	
Daytime % of total meals	53	44	31	43	0	
Nighttime % of total meals	47	56	69	57	100	
Meal size (g)	77 ± 5	146 ± 2	227 ± 7	308 ± 5	375 ± 25	
Pre-meal time (min)	123 ± 16	149 ± 23	160 ± 26	193 ± 51	308 ± 173	
Post-meal time (min)	111 ± 15	171 ± 26	213 ± 31	168 ± 32	120	
Insulin						
% of total meals	56	21	18	2	2	1
Daytime % of total meals	41	52	33	0	50	100
Nighttime % of total meals	59	48	67	100	50	0
Meal size (g)	75 ± 3	146 ± 3	225 ± 6	313 ± 13	400	
Pre-meal time (min)	141 ± 17	165 ± 35	180 ± 26	198 ± 33	265	
Post-meal time (min)	168 ± 17	150 ± 30	148 ± 28	225	105	

Table 11. Predicted daytime and nighttime meal size in concentrate fed reindeer using energy deficit (ED) and rumen deficit (RD) equations from this study. For definitions see Materials and Methods.

	Control (n = 4)		Insulin (n = 4)	
Models	Day	Night	Day	Night
observed	**127 ± 14**	**174 ± 14**	**134 ± 13**	**130 ± 11**
Meal size (g)				
ED	100	256	136	119
RD	-100	190	107	95

General Conclusion

This thesis addresses short-term regulation of appetite of reindeer during winter and explores the role that insulin plays in daily meal patterning, voluntary food intake (VFI) and subsequent effects on body mass and body fat. Winter was chosen because the seasonal food intake cycle of reindeer is anticipatory of seasonal food nutrient availability and of the adverse energy budget of winter. This study confirms that voluntary food intake in winter is highly regulated even when food is made freely available. This study shows for the first time that there is a tight linkage between energy intake and energy requirements on a meal to meal basis (Chapter 2). Other studies show that body mass undergoes only a modest decline throughout winter (McEwan and Whitehead 1970; White et al. 1984).

Feeding activity in reindeer was different day and night and, as proposed elsewhere, feeding activity is likely modulated by photoperiod (Loudon 1994), transduced to the brain by amplitude and duration of nocturnal secretion of the pineal hormone melatonin (Eloranta et al. 1990). Since peripheral and central signaling systems are implicated in the regulation of food intake and since reindeer exhibit pronounced seasonal fluctuations in serum insulin levels (Larsen et al. 1985), I proposed a role for insulin in regulating meal patterns possibly through its relationship with glucose homeostatis. Glucose homeostasis is the basis of one of the oldest theories proposed in the last century explaining food intake regulation, namely the glucostatic theory of Mayer (1953). Thus, reindeer could provide a ruminant model to test this theory.

Two techniques were developed, the first was one to estimate body fat *in vivo* and the other was a means to measure meal size and the time interval between meals over a

24 h period. Backfat depth (BFD) proved to be useful as a predictor of total body fat in the reindeer used in this study (Chapter 1). I found the accuracy in measurement of BFD with the ultrasound technique was 0.1 cm and was within the accuracy needed to detect significant physiological changes in a live animals. Identical trends in body fat were observed using BFD and body condition score, the predictor that had been validated and applied to field studies (Gerhart et al. 1996).

Measuring meal size and the interval between meals over 24 h periods allowed me to determine aspects of feeding behavior that are well known in laboratory animals, including domestic sheep (Campling 1970; Baile 1975), but have heretofore not been reported in north temperate deer. Thus, I contrasted meal patterning in a species that is essentially nychthemeral in feeding (the reindeer), with published data on the domestic sheep, which is diurnal. The very close meal size versus pre-meal interval relation of reindeer and sheep during daytime feeding, suggested similarly structured physiological controls over feeding behavior in these very different ruminants.

Voluntary food intake of reindeer fed concentrate ration during December (Chapter 2) was down regulated to small, regular meals during daylight and fewer but larger meals during nighttime. Daytime meal size approximated the energy deficit incurred since the previous meal, inferring a metabolic control over intake and suggesting a role for insulin and glucose in food intake. The occasional oversized nighttime meals suggested periodic deregulation of appetite: and dominance of a food-processing model operating at night. I suggest that the secretion of melatonin could be inducing the large nighttime meals and also may change the animal's sensitivity to insulin. I theorize that melatonin allows, or permits the release of a "brake" on the appetite center resulting in

intake of large meals. Insulin could be the hormone acting as a "brake" on the appetite center. This constitutes an intriguing new concept for intake regulation in ruminants and needs to be tested in other ruminant species. The advantage of large nighttime meals should be that they result in long digestion periods that would enhance foraging efficiency during months of nearly 24 h darkness. A combination of large nighttime meals and seasonally reduced metabolic rate should minimize negative energy balance throughout the winter and thus allow reindeer to maintain their fat reserves in an energetically challenging environment. Particularly for pregnant reindeer, sufficient energy stores must be maintained to drive late fetal protein deposition and to initiate lactation before adequate food is available.

I was able to demonstrate the existence of a rhythmic short-term pattern in serum insulin concentrations and hence in insulin secretion (Chapter 3) during fasting in the reindeer. Since the insulin oscillations were not correlated with those for serum glucose and lactate, I concluded insulin secretion was not a product of rumination bouts that continue for over 4 h following feeding. The periodicity of insulin secretion was of similar duration as the inter-meal interval for reindeer fed a concentrate ration during winter (Chapter 2). Hence, I hypothesize that periodic insulin secretion could be playing a role in meal initiation.

Chronic injections of long-acting insulin resulted in regular meal eating throughout the 24 h periods, i.e., differences between daytime and nighttime meal patterns disappeared (Chapter 4). Based on these findings I proposed that a combination of insulin secretion and sensitivity to insulin results in the daytime and nighttime differences observed for feeding behavior of reindeer in winter. Since the main effects of

insulin on feeding behavior were restricted to nighttime feeding, I speculated that melatonin was responsible for decoupling the daytime meal size versus meal interval-meal relationships; i.e. the animal becomes less responsive to serum insulin concentration and loses its feeding rhythm.

A combination of rhythmic variation in satiety response to meals during daylight and decoupling of meal size and frequency at night is suggested as an endocrine model underlying daily appetite regulation in the reindeer. Such a model should be adaptive in north temperate species as it allows for fewer feeding events at night and should lower daily maintenance energy requirements during winter.

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Appendix 1

Simulation model: Modeling of meal size and intermeal interval in reindeer. Data are for Chapter 2.

Simulation modeling

We built two models to evaluate how closely individual animal data sets agree with meal size and feeding frequency is relation to an energy deficit incurred since the last meal (metabolic model), and meal size and feeding frequency is related to food rate of passage estimated as a deficit in rumen fill since the previous meal(physical model).

The models were deterministic and each simulation was initiated by an estimate of the pre-meal interval. Model output was meal size and inter-meal interval. We used an assumed, derived, or randomly assigned estimate of the pre-meal interval to calculate meal size through successive feeding bouts. Outputs from the model were calculated through time using a spreadsheet (Microsoft Excel Version 2000). Cyclical calculations of meal size were continued until the meal size went to zero, stabilized or increased to a size greater than that recorded in this study (550g DM). From the theoretical meal size and inter-meal interval a calculation was made of the expected number of daily feeding events and the theoretical DDMI.

(i) *Metabolic Model* (MM): This model tests the theory that each meal makes up the energy deficit incurred since the last meal. ED (Y) was predicted from pre-meal interval (X), $Y = 0.42 \bullet X$. The ED was used to predict meal MEI using eq.13b, or 13c if pre-meal interval was > 200 min. We then developed a regression equation relating post-meal interval to meal MEI. We calculated post-meal interval (Y) using the equation: $Y = 0.2535 \bullet X + 130$ where X = meal MEI. This estimate of post-meal interval then

became the pre-meal interval to calculate ED for the next iteration of the feeding cycle. To compare model outputs, each meal MEI (kJ/ $kg^{.75}$) was converted to meal size (g DM) assuming a mean body mass of 104 kg and a food metabolizability of (13.07 kJ/g DM). Although meal size was the main model output, time intervals between meals also was compared with those of the study and with those predicted through iterative steps of the other models.

(ii) *Physical Model* (PM): This model was a test of theory that each meal equaled the amount of dry matter disappearing from the rumen since the last meal, i.e. the rumen deficit (RD). Pre-meal interval was used to predict a RD based on rumen outflow (eq. 6, 7, 8) and expected DM disappearance over this time period. RD then gave an estimate of meal size (using eq.15b). The meal size post-meal interval relationship (eq 12d), then determined the time of next feeding. We developed a regression equation relating post-meal interval to RD. RD (Y) was calculated by the equation $Y = 0.002 \bullet X$ where X = post-meal interval.

Comparison of data with model output: In addition to determining the number of cycles required to stabilize meal size and inter-meal intervals, the models were also compared with 24 h data, stratified by day and night, for three individual reindeer (Larkspur, Mimi, Tamarack) for which complete data sets allowed comparison.

Results

Simulations using MM and PM

Point of stability was reached after 4 -7 iterations for both models giving inter-meal intervals of 148 and 149 min (Table 12) (Fig.30a-d), and resulting in a theoretical 10 meals daily. Meal size was predicted to be between 179 and 189 g (Table 12) (Fig.30a-d). The PM predicted 8 and 10 g larger meal size than MM. For these models DDMI ranged between 1.79 and 1.89 kg. Comparison of meal size and meal interval of the theoretical with the empirical results (Table 12) suggest (i) MM underestimate meal size by 17 % and meal interval by 2%, (ii) the PM overestimate meal size by 71% and meal interval by 9 %. Mean DDMI from model predictions (0.61-0.71 kg) were less than measured (1.18 ± 0.08 kg). Post-meal time and RD when predicted by the PM were 0.9% greater than those for the MM. Model predictions of meal sizes were close to measured values. Significant correlations were found between predicted and measured meal sizes on the three individual reindeer for all models with the exception of the MM for Larkspur (Table 12). Meal sizes for each reindeer were close to daytime meal sizes (e.g. Fig. 31, Larkspur). The relationship between predicted nighttime meal sizes for three reindeer was highly variable. Comparison between model outputs suggest that the ability of MM to predict small daytime meals is better than with the PM. Inter-meal intervals of >100 min were more accurately predicted than those <100 min (e.g. Fig. 132, Larkspur)

Discussion

In the MM and PM simulation, the inter-meal interval is set by empirical data. Thus, the actual level of ED or RD does not initiate the feeding event: i.e. the models do not test the actual sensor. It is not surprising therefore that successive estimates of meal

size do not result in a drift to inordinately small or large meal sizes. The models show that when an inter-meal interval is imposed, the MM model results in smaller meal sizes than the PM, but that the model MEI > ED (17 %) and the meal size < RD (62%). The observed greater variability in predicted nighttime meal sizes in our model runs support the idea of a diurnal phenomenon in regulation of DDMI. Nothing in the modeling or our feeding behavior observations accounts for the occurrence of these few large meal sizes. However, our modeling suggests that the PM could account for the long post-meal intervals following the large feeding bouts at night. A dominance of the MM during the day results in short feeding bouts and small meal sizes.

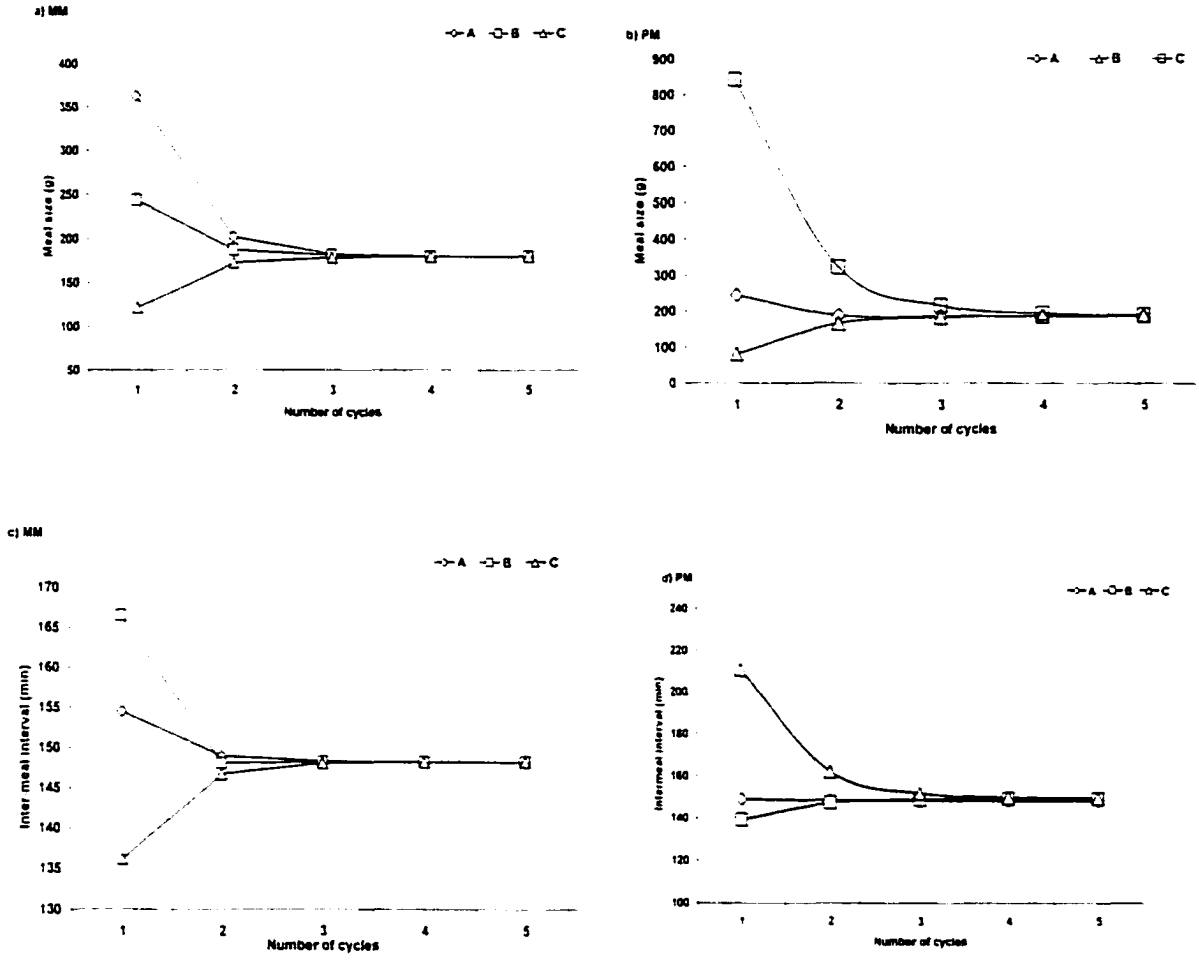

Fig.30. Simulation model of successive estimates of meal size (a. b) and intermeal interval (c. d) as generated by metabolic model (MM) and physical model (PM) using different starting values.

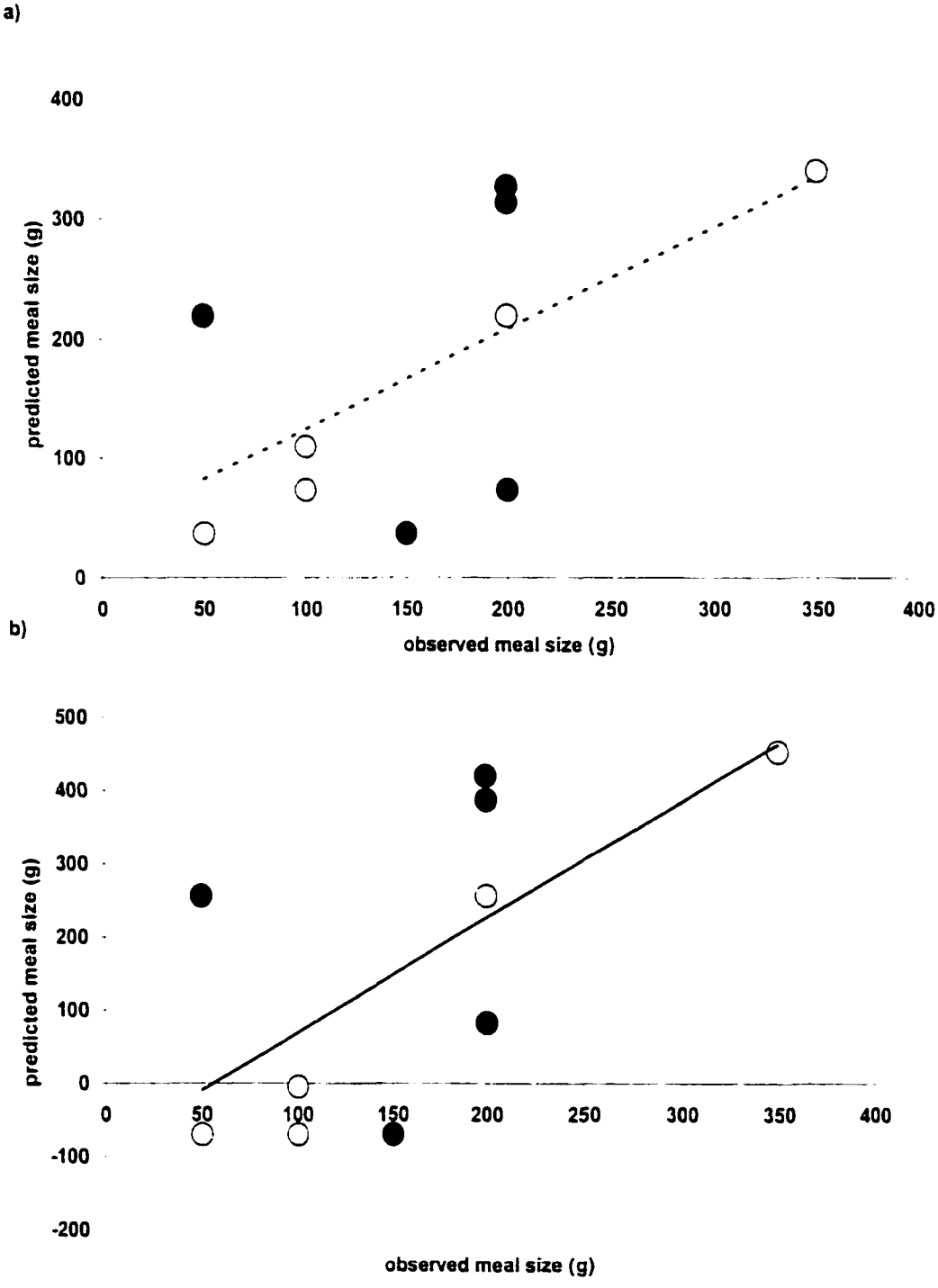

Fig. 31. Example of meal size predicted from the MM (a), and PM (b) in comparison with observed meal size for Larkspur at LARS, Alaska 1996-1997. Meals are separated as daytime (o) and nighttime (•). Regression lines represent line of best fit to all data; Correlation analysis is shown in Table 13.

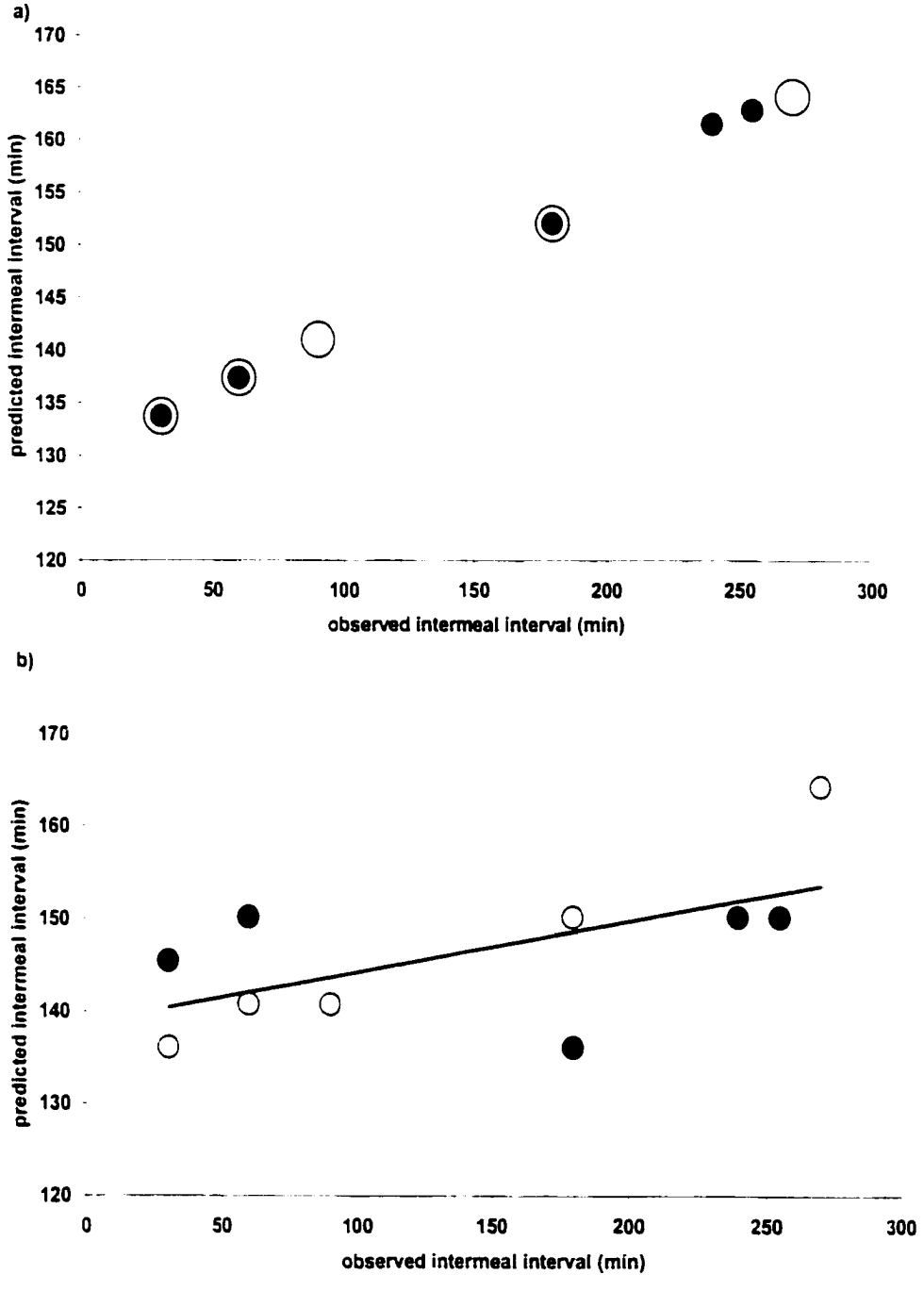

Fig.32. Example of inter-meal interval predicted from the MM (a), and PM (b) in comparison with observed inter-meal interval for Larkspur at LARS, Alaska 1996-1997. Meals are separated as daytime (o) and nighttime (•). Regression lines represent line of best fit to all data; Correlation analysis is shown in Table 13.

Table 12 . Comparison between model (MM and PM) generated and measured meal size and inter-meal interval for concentrate fed reindeer (n=8) at LARS, Alaska 1996-1997.

Type	Measured		Model	
	Meal (g)	Interval (min)	Meal (g)	Interval (min)
Metabolic (MM)	181	148	151	145
Physical (PM)	189	149	323	162

Table 13. Correlations between predicted and measured meal sizes for three individual reindeer at LARS, Alaska 1996-1997.

Animals	Metabolic Model (MM)	Physical Model (PM)
Larkspur	r^2=0.38 ; P=0.056	r^2=0.44 ; P=0.036
Mimi	r^2=0.87 ; P=0.002	r^2=0.78 ; P=0.008
Tamarack	r^2=0.59; P=0.025	r^2=0.59 ; P=0.026

Appendix 2

Absolute serum glucose and lactate concentration in reindeer fasted 18 h. Data are for Chapter 3.

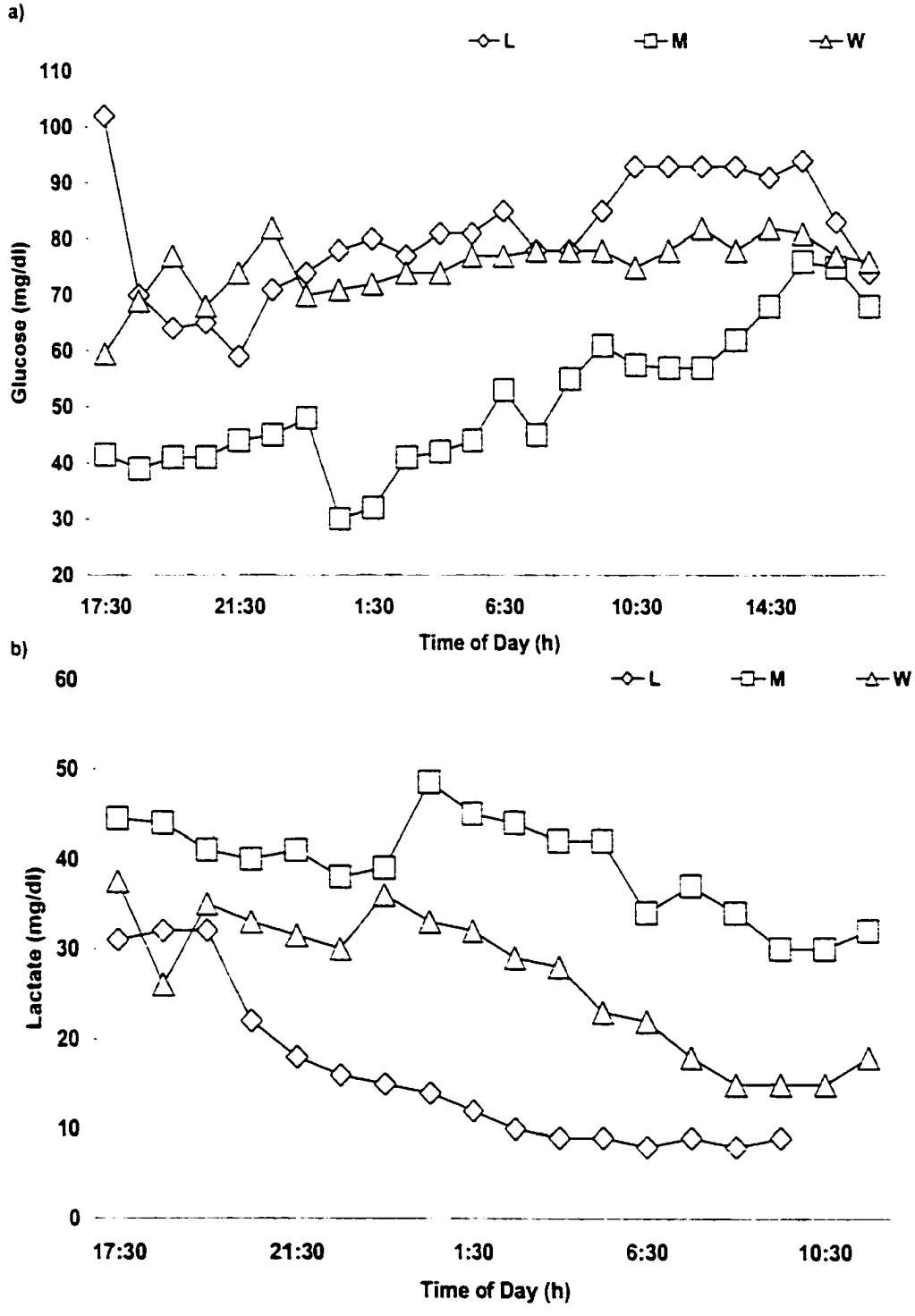

Fig. 33. Serum glucose (a) and lactate concentrations (b) in three reindeer during an 18 h fast. 17:30 (zero hour) for sampling.

Appendix 3

Absolute measures of hormone and metabolites, body mass and back fat depth, daily dry matter intake in control an insulin treated reindeer. Data are for Chapter 4.

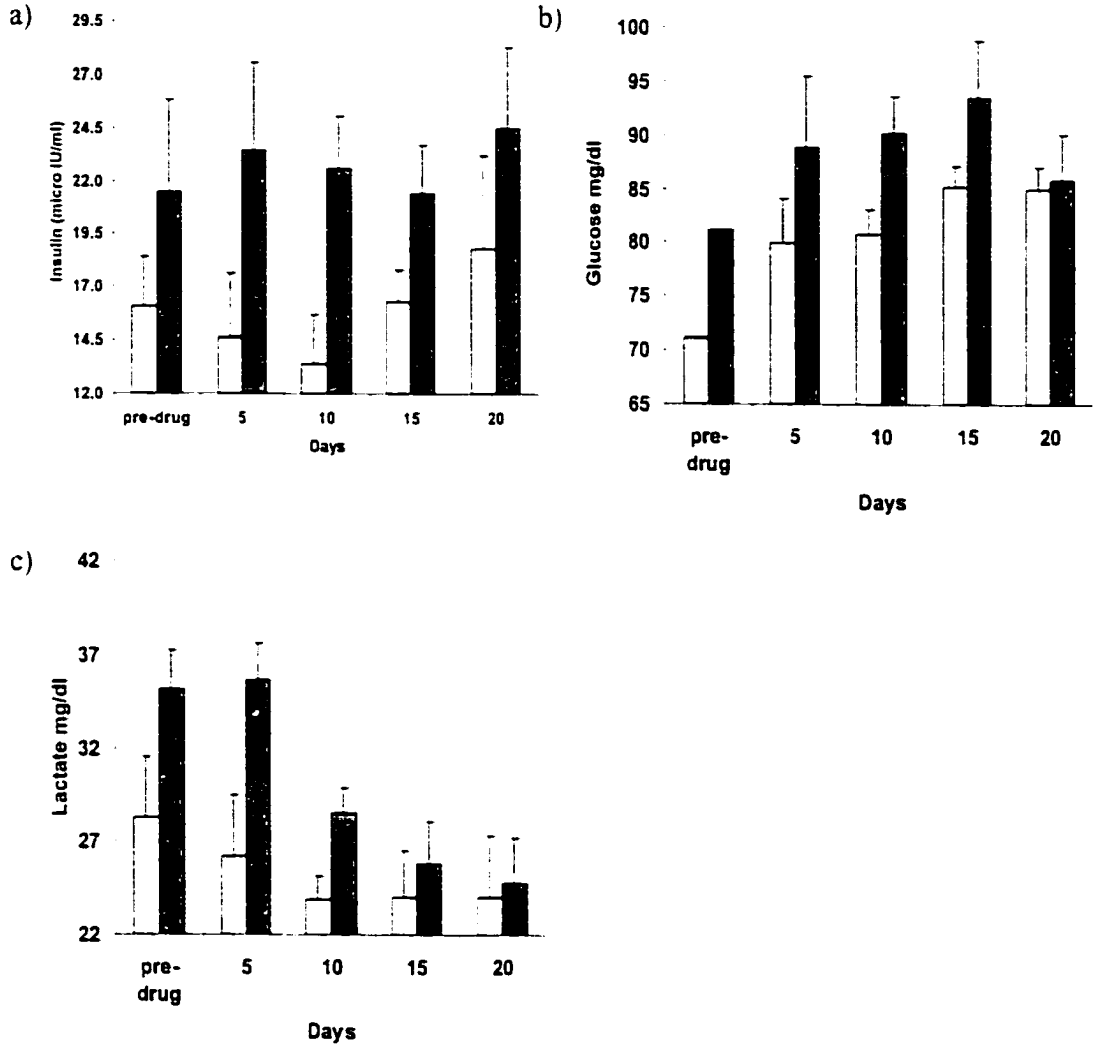

Fig. 34. Serum insulin (a), glucose (b) and lactate concentrations (c) for control (open bars) and insulin treated (filled bars) reindeer over a 21d treatment period. Data are expressed as mean ± SEM.

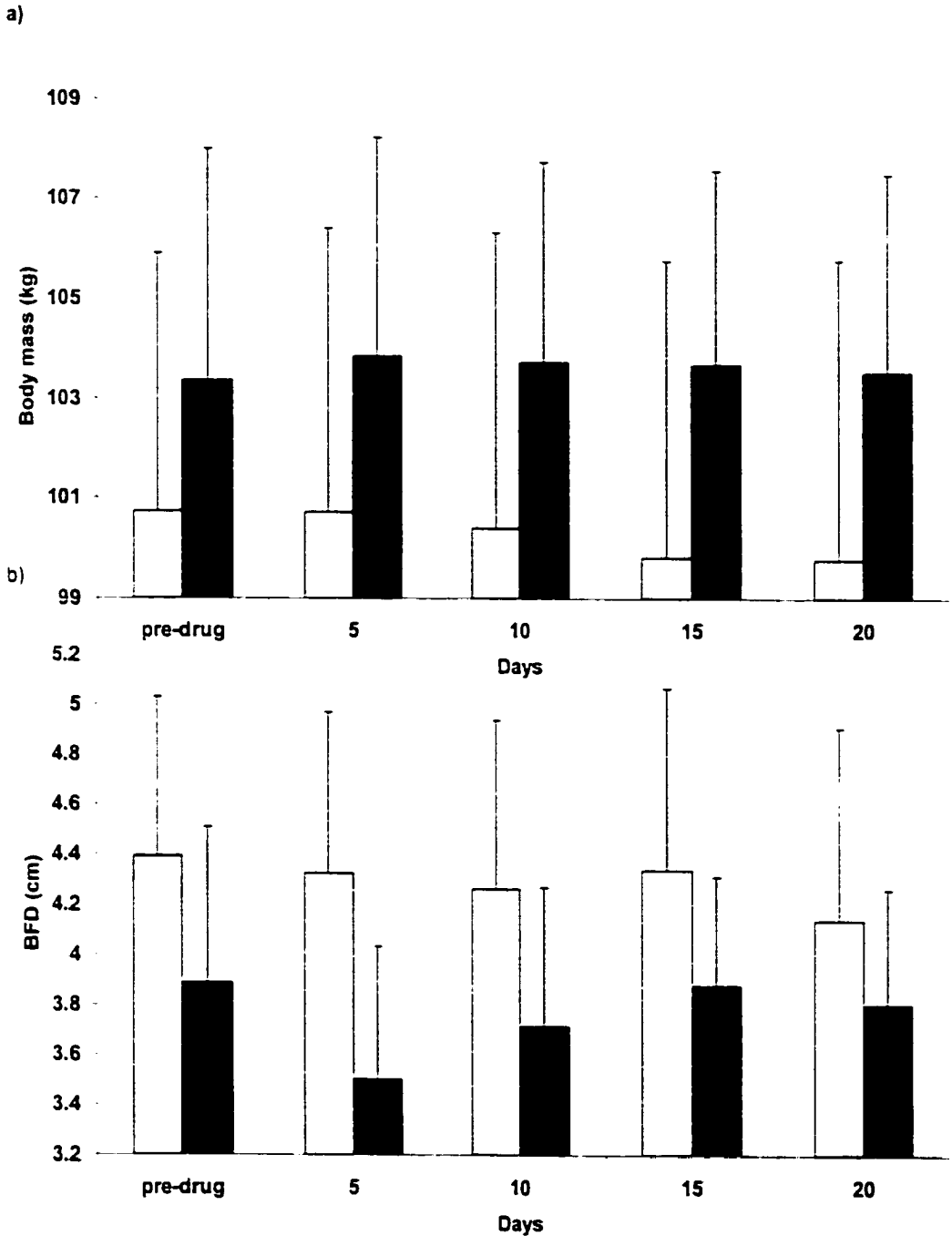

Fig. 35. Body mass (BM) and backfat depth (BFD) response in control (open bars) and insulin treated (filled bars) reindeer over a 21d treatment period. Data are expressed as mean ± SEM.

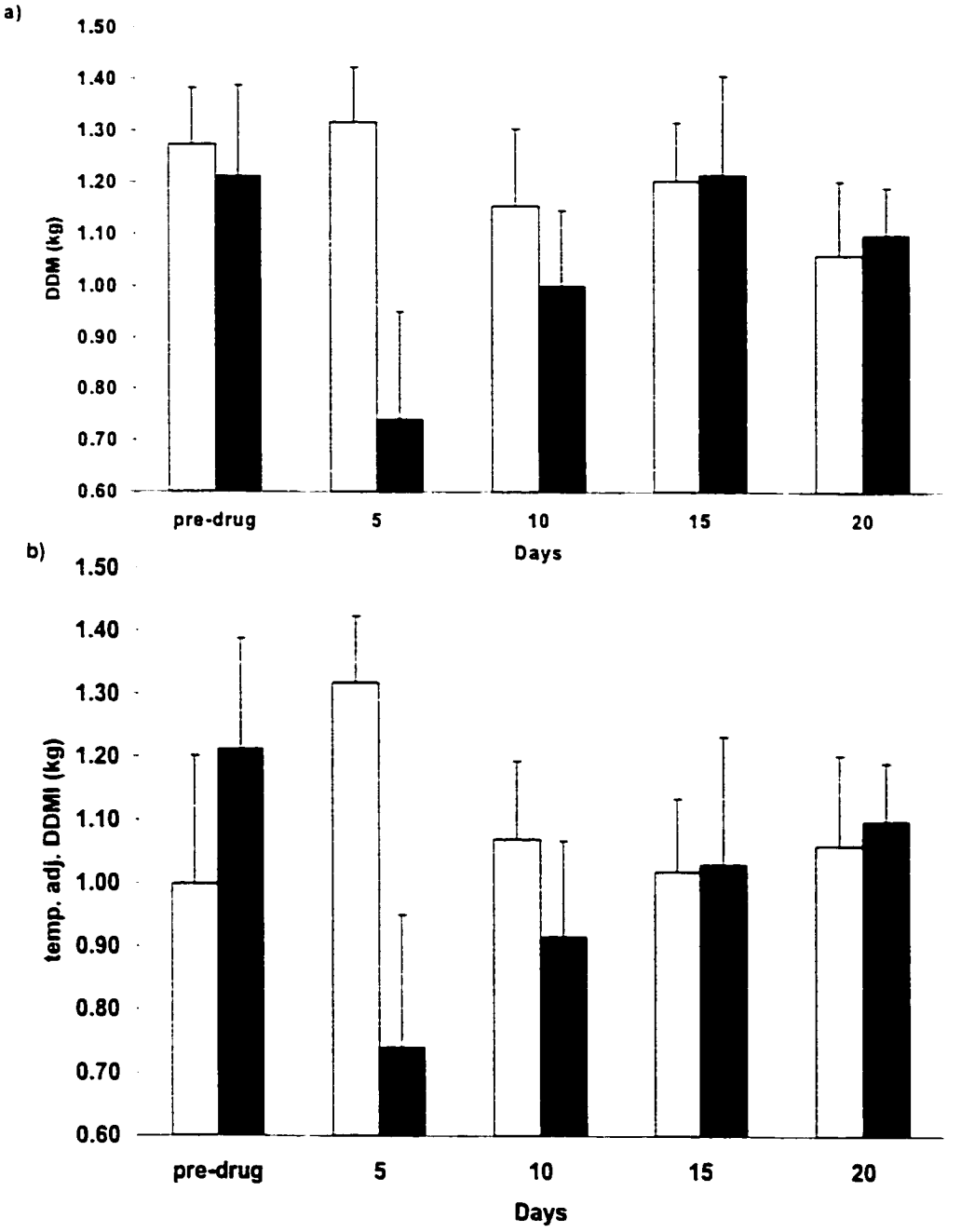

Fig. 36. Daily dry matter intake (DDMI) (a) and temperature-adjusted DDMI (b) response in control (open bars) and insulin treated (filled bars) reindeer over a 21d treatment period. Data are expressed as mean ± SEM. See materials and methods for definitions.